

nature

11 October 1979

Cambodia's long path back

Relief operations in Cambodia must be based on accurate information, of which there is little. And a new scientific and medical community will have to be developed.

AT LONG last there seems to be some movement on the diplomatic front over the tortured question of aid to Cambodia. Announcements of a British government provision of more than £4 million in medical and food aid, together with news that Oxfam is starting to take supplies into the country, do at least represent a step forward from the days of wretched international diplomacy in which the precise status of Cambodia's two claimants to power seemed a matter of more concern to the Western countries most capable of helping than did the fact that millions were starving to death. But now in the euphoria that at least something is happening in the short-term, attention must not be diverted from the long-term needs of the area.

Cambodia, or Democratic Kampuchea as it has recently been called, is a very fertile area. The story is told by Prince Sihanouk that US AID officials once visited Cambodian villages to encourage the planting of higher yield varieties and came back the next year not to find bumper harvests but that the villagers were now people of leisure, being able to fulfil all their needs on only half the land. All the more galling then that the successive disruptions of the past ten years — the US bombing, three years of the Pol Pot regime and then the Vietnamese invasion during the planting season in late 1978 — should have brought the country to its present position.

The problem with dispatching aid in the past few months has been the legitimacy issue. Vietnam-supported Heng Samrin has driven the Pol Pot forces into the more inaccessible parts of the country, and by the general rules of diplomacy (as applied for instance to Iran and Uganda) would probably qualify for recognition. But Pol Pot is supported by China, whereas Heng Samrin ultimately receives support from Moscow. And at present China is being courted by so many countries that when the question of recognition of a Cambodian government came up recently, the United Nations voted strongly in favour of seating the Pol Pot regime.

Thus, when the country desperately calls for international assistance there is general confusion. Cool logic suggests that the aid should go by way of Phnom Penh, the capital in the hands of Heng Samrin. But the involves at least some sort of acknowledgement of Heng Samrin's legitimacy — particularly difficult for Britain to swallow since Mrs Thatcher made it clear that she would do nothing to help Vietnam. There are, furthermore, always those who can derail a relief operation by

claiming that the assistance will simply fall into the hands of the army, which some of it undoubtedly will.

Mercifully the British government has at last made a substantial step forward, and other countries seem in the process of doing so also. The question then remains of how this avalanche of aid will be put to good use. Observers of the country stress how little is really known of Cambodia's plight. Even the population is a matter of conjecture, estimates varying between 3 and 6 million. Such vital information as the prevalent type of starvation, and the distribution of people — are they in relief camps around the cities, or in scattered villages? — is barely known. And yet the character of relief operations depends crucially on this knowledge. One wonders, parenthetically, whether the United States and Soviet Union, always prepared to launch a satellite to keep an eye on other people's troubles, have an adequate supply of pictures to answer many of the questions concerning population distribution, how they are proposing to make this information available, and if not, why not.

If there are short term problems of the intelligent deployment of aid, there are, equally, long-term problems for the country. To regain its status as the rice bowl of Asia, a vigorous agricultural policy will have to be evolved in the next few months. The last planting, in war-time conditions, only used 10% of the available land — getting back to more normal land usage will not just happen automatically. Nor will medical services revive without much assistance. Estimate of the number of doctors who have survived range between 40 and 50 — a tenth of the figure of a few years ago.

If Cambodia's agricultural and medical community is to be rebuilt properly, a major international operation must be set up very soon to speed the training of qualified manpower. This will need concerted effort by many nations; it is to be hoped that the diplomatic and political considerations which have bogged down relief plans will not prevent institutions in the West from helping in the recovery of a shattered country.

● While governments are beginning to act, there is still enormous scope for charities with their greater flexibility. A UK appeal through the normal channel of the Disasters Emergency Committee has not met with a very generous response. Two charities which do seem to be capable of responding to local needs are Oxfam, Banbury Road, Oxford, and Medical Aid for Cambodia, 36 Wellington Street, London WC2. □



Military in clash over US nuclear fusion research

US physicists claim that the use of heavy ion beams is now a leading contender for commercial fusion technology — but that research efforts are being hampered by the aspirations of the military establishment. **David Dickson** reports

TENSIONS are growing between the Carter administration and the US Congress over the appropriate strategy for pursuing one of the most promising paths towards a commercially-viable nuclear fusion energy programme — namely the use of inertial confinement techniques, which would harness the energy released by fusion during the implosion of a small hydrogen pellet hit by a converging beam of immense power.

The prime cause of this tension is a dispute over whether, since such techniques can be applied to the detonation of nuclear weapons, all research on inertial confinement should be conducted as a military project, or whether the potential civilian applications would be better served through a separate non-classified programme.

Behind this is a more conventional dispute involving competition over access to and control over limited research funds between different national laboratories. Here the current control of inertial confinement research by the two weapons laboratories run by the University of California under contract from the Department of Energy — the Lawrence Livermore Laboratory and the Los Alamos Scientific Laboratory — is being challenged by physicists from some of the major accelerator laboratories. In particular, these include the Argonne National Laboratory in Illinois, the Brookhaven National Laboratory on Long Island, and the Lawrence Berkeley Laboratory in California.

The physicists are particularly concerned that if, as Congress is now demanding, one of the weapons laboratories is designated the lead laboratory for inertial confinement research, this could penalise their own efforts — even though, they claim, these may have the best chance of achieving commercial viability.

Research has been carried out since the early 1960s at the two weapons laboratories on the possible use of laser beams to ignite the hydrogen pellets — a path to fusion which is beginning to compete with more conventional programmes, such as the magnetic confinement techniques being studied at Princeton University.

Work at Lawrence Livermore has concentrated on the use of glass lasers, and has been carried out on a variety of machines of increasing power, the construction of the latest, NOVA, having begun this summer. At Los Alamos, research has been conducted in parallel on the possible use of carbon dioxide gas lasers, and the laboratory is also con-

structing a large experimental machine known as ANTARES.

Within the past few years, however, high energy physicists have begun to explore a different approach, using particle beams to provide the necessary 'driving' impact on the pellet. In particular they have considered the use of heavy ions created by the particle accelerators already in use for basic physics research. (See *Nature* 276, 19-23; 1978).

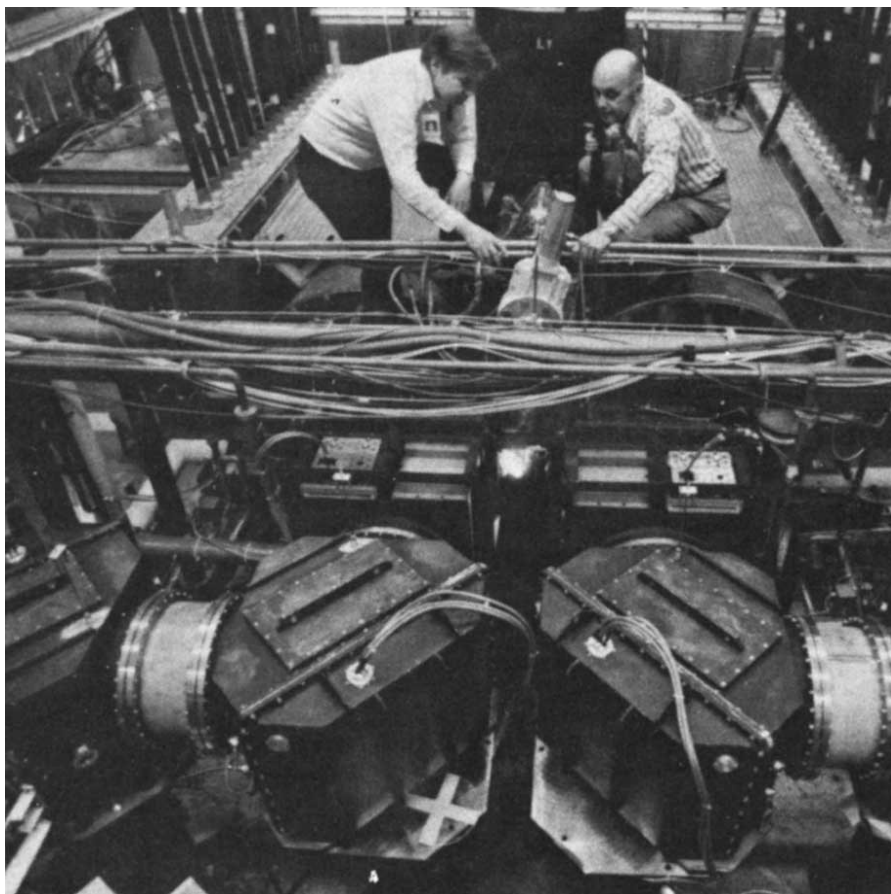
These physicists claim that two reports — both as yet unpublished — support their contention that there is growing disenchantment with the use of lasers for commercial fusion technology, and that particle beams in general, and heavy ion beams in particular, may offer the most practical solution. But they also claim that since heavy ions have no direct military applications — there is no way to put an accelerator within a nuclear weapon — their research is not receiving the support it merits.

Strong support for the commercial prospects for heavy ion beam research is said to come from a report prepared for the Department of Energy by Dr John Foster,

Vice President for Research at TRW Inc. Although the full report is yet to be published by the DoE, it is said to back-up Dr Foster's statement to the department's Energy Research Advisory Board in May that he considered the use of heavy ion beams to be the "first candidate" for a conservative approach to a workable fusion technology.

The second report has been prepared for the Electric Power Research Institute by a committee chaired by Dr Keith Brueckner, of the University of California, San Diego. Dr Brueckner's report does not make any comparison between the various driver technologies now under study; but it is said to confirm the Foster committee's view that there appear to be no insurmountable obstacles to the use of heavy ion technology. "The current level of funding for heavy ion beam research certainly seems to be incompatible with its promise as a commercial technology," Dr Brueckner said last week.

Both of these reports are now being considered by the Department of Energy as it prepares its budget submissions for fiscal year 1981, which President Carter will submit to Congress next January. And Dr Frank Press, director of the Office of Science and Technology Policy, is said to be among those who have been pushing the department to develop a separate civilian inertial confinement research programme,



Laser research at Los Alamos — but are they off beam?

presumably with a greater emphasis on particle beam research.

Such moves, however, are currently being resisted by Congress, and in particular by the House Armed Services Committee, members of which argue that most of the physics of pellet design and energy transmission yet to be understood is common to both military and civilian needs. There would therefore be no need yet to split the two aspects of the research — indeed this could lead to unnecessary duplications of effort.

In passing the DoE's budget request for 1980, the committee therefore directed that the whole of the inertial confinement programme should remain conducted through the weapons laboratories — and added the rider that no money should be provided for specific studies of the commercial applications of inertial confinement fusion, or for "activities which will not be carried out in direct support of the ICF programme leading to military applications."

The committee's feelings were reflected in its reaction to the DoE's budget request. Initially, the department had requested that for 1980, about \$3.8 million out of a total budget for inertial confinement research of \$146 million should be allocated to heavy ion beam research at the accelerator laboratories.

Following the recommendations of the Foster committee, however, which both pointed out some of the technical limitations facing the expansion of the laser programmes and recommended a major increase in funding for particle beam research, the department suggested some rearrangements in the fusion research budget which would have increased the amount spent on heavy ion beam research to between \$12 and \$15 million. The suggestion was rejected by the House Armed Services Committee, however, which cut the request back to little higher than its original level.

Keeping options open

Scientists at the two weapons laboratories are still confident that they are justified in concentrating their attention on the physics of glass and gas lasers — even from the point of view of eventual civilian energy production. "It's much too early to make any assessment about the relative merits of the different systems. These are experimental programmes, and progress will only be made through experiments," says Dr Charles Fenstermacher, programme manager for the advanced driver development at Los Alamos.

"There are many problems in the physics that are not yet understood. And you do not want to drop any option until it is certain that it is not an option."

Most physicists agree with both the DoE and with Congress that it is likely to be several years before a clear idea emerges of the most practical direction for inertial

confinement as an energy technology. The problem is that, for each of the possible alternatives, generating the data required to make such an assessment involves considerable capital outlay — and therefore results in competition for relatively scarce funds.

Already Livermore's NOVA, which had originally been scheduled for completion in 1983, and for which Congress had authorised the full construction costs of \$195 million, has been cut back following the recommendations of the Foster committee, and is now proceeding in two stages, construction of the first of which started in May.

Physicists at the accelerator laboratories hope to catch up — and possibly to pass — the weapons laboratory researchers in two stages, first by carrying out a joint accelerator technology demonstration programme to show the feasibility of their ideas, and subsequently, if this is successful, to move to a larger-scale machine capable of carrying out target experiments.

The first step in this process would include a substantial commitment to heavy ion beam research in the 1981 budget, possibly in the region of \$20 million, with the bulk of the funds going to Argonne and Berkeley, and the remainder distributed between the other laboratories.

The next stage would obviously be more expensive. Designs have already been produced for a heavy-ion demonstration experimental facility (HIDE), which would be capable of reaching energies of 100 kilojoules, but could cost more than \$100 million (and theoretically it might make more sense to go directly to an even bigger machine, costing about \$500 million).

Shortage of funds could, furthermore, be accompanied by a shortage in qualified research scientists. The House Armed Services Committee points out in its report that "since this is a relatively new technology, the supply of such people is small"; accelerator scientists argue this may be one reason the weapons laboratories are reluctant to see a proliferation of inertial confinement research programmes outside their control.

They also claim that the fact that much of the current research must remain classified is one hindrance resulting from a military-controlled programme — arguing that it should still be possible to deal with the classification of potentially sensitive details even within a programme directed outwards non-military goals (as happened, for example, in the early stages of the nuclear power programme).

But whether the administration will be able to persuade Congress of its claim for the need for a separate civilian inertial confinements programme, in the face of a military establishment which sees such a programme as undercutting its own interests in such research, remains an issue that will be fought out bitterly in the months ahead. □

Congress boosts spending on health research

THE US Congress has agreed to increase the research budget of the National Institutes of Health by 8.2% to a total of \$3.4 billion for fiscal year 1980. This is despite President Carter's contention in his budget request at the beginning of the year that since the NIH had received a budget increase of almost 25% in the current year, no further increase was necessary.

In discussing their separate versions of the Department of Health, Education and Welfare's Appropriations Bill, representatives in both the Senate and the House agreed to a total NIH budget which includes recommended increases that had previously been made by both sides. In particular, this will mean that for the first



Representative Boland: dispute over Galileo

time the budget of the National Cancer Institute will exceed one billion dollars next year. It will also mean an increase of \$26 million, as suggested by the House, for the National Institute of Arthritis, Metabolism and Digestive Diseases.

In a separate action, Senate and House conferees agreed on a budget for the National Science Foundation of \$996 million dollars for 1981. Although less than the President's request for \$1,006 million, this still represents an increase of 9.3% over the current year's budget.

The conferees also agreed on a budget increase of 8.2% for the National Aeronautics and Space Administration, including a 9.9% increase as requested by the Administration for research and development. Final action on NASA's budget, however, has been held up by a row between the two legislative bodies over future funding for Project Galileo, a proposed two-spacecraft mission to Jupiter.

The House, at the recommendation of representative Edward Boland, wants its appropriations committee to have the power to veto any proposals to split the mission — which may be necessary as a result of delays to the Space Shuttle. The Senate has refused to accept the condition and NASA's 1980 budget has been held up. □



Uranium strip mining: "like going to the Moon"

Unhappy hunting grounds: the Indian end of nuclear energy

Joe Schwartz talks to Winona LaDuke and Herb Blatchford of the American Indian Environmental Council about the hazards of uranium mines in native lands

"THEY start by taking core drills. Then the rigs move in, next to a house or a corral, it doesn't matter to the companies. The pumps spray radon saturated water out on the ground where it forms radioactive pools. Animals drink it. Children play in it. The dogs become listless. The children sleep all night without resting. They complain of nausea. Dietary problems and gastro-intestinal complaints are common."

Herb Blatchford, 51, and Winona LaDuke, 20, of the American Indian Environmental Council (AIEC) were in London last week for a short stopover on their way to the Copenhagen Conference on Uranium Mining and Indigenous Cultures on 18-20 October. "It's an international conference of irradiated natives" says Winona LaDuke drily. But, with most of the world's uranium mining taking place on the lands of native people, Blatchford and LaDuke have a lot of first hand experience about what goes on at the "front end" of the nuclear fuel cycle. SCRAM, the Scottish coalition of environmental groups, had invited them to Edinburgh to discuss the planned uranium mine in the Orkneys that will fuel the Torness power plant (20 September, page 171).

"The disruption is unbelievable" says Blatchford. "In fact it's so bad that Nixon's Project Independence in 1973 called for 'National Sacrifice Areas.' The Diné (Navajo) lands in the New Mexico and Arizona were to form the southern end of an energy corridor extending up through Wyoming, South Dakota and into Saskatchewan that would end up a total wasteland."

"The largest uranium strip mine in the world is located at Laguna Pueblo near Albuquerque. It's owned by Anaconda. Going there is like going to the moon."

People are listless, the animals show no motion, there are machines grinding all the time. The mine covers the whole valley and the Rio Paguete is a fluorescent green. Originally it was farming community."

Energy development in the American Southwest is intense. Navajo lands at present house four coal mines, five coal fired power plants, 36 operating uranium mines, six operating uranium mills and six abandoned uranium mills. Winona LaDuke describes how these arrangements are negotiated. "The leases are negotiated through the Bureau of Indian Affairs in consultation with tribal councils. The tribal councils are a creation of the BIA, the usual form of native government being a council of elders with decisions taken by consensus. The tribal councils are funded by the BIA. In the Navajo lands there are 74 members of council earning \$13,000 a year. The average Navajo income is \$1,400 a year."

"Royalties are minimal. The present rate of \$0.6 a pound for yellowcake is a royalty of 1.2% of the market price of \$50 per pound. You have to compare this to the standard royalty of 12% paid to the Bureau of Land Management and the Federal Parks for resources taken out of other federal land. The royalty is paid to the Area Director of the BIA and is given out only with the approval of the Secretary of the Interior. We are legally wards of the government not beneficiaries so we have to beg back the money that is supposed to be ours."

Court cases to change the legal status of the Indian nations or to alter present contracts have failed. Last spring the Jicarilla (Apache) in Northern New Mexico managed to win a tax case meaning that they will no longer have to pay federal tax on their royalties. LaDuke says this is unique to native communities in the world.

"There's nothing compared to it. It's a system of involuntary servitude. For example, in one year the Diné (Navajo) nation exports enough energy to provide the needs of the state of New Mexico for 32 years. But 85% of Navajo households don't have electricity. This makes the Navajo nation an energy colony."

But finally, it is the hazards of uranium mining itself that is the urgent issue. "Eighty five per cent of the released radioactivity remains in the tailings. Rainfall tends to release the Rn222 which in New Mexico rises to the Continental Divide and then settles back down collecting in basins. We are starting to see long range effects now."

After a major expose in the *Washington Post* last year, the US Public Health Service attempted to locate some 250 Navajo and 400 white miners who had worked the Red Rock Cove mine near Shiprock operated by Kerr-McGee. Of 100 miners eventually surveyed, 25 had died while still in their 40s and an additional 20 were found to have cancer.

The environmental prognosis for non-miners appears to be poor. A Los Alamos report (*Uranium Mill Tailings, Environmental Implications*, February, 1978) was pessimistic about the possibilities of controlling radon release. "Our research indicates that 12 feet of clay are required to reduce the radon exhalation rate by 99% and the remaining 1% is still about four times the typical soil exhalation rate."

"Perhaps the solution to the radon problem is to zone the land into uranium mining and milling districts so as to forbid human habitation."

In spite of the demonstrable hazards and what Herb Blatchford calls the "ultraexploitation" of native peoples there is going to be opposition to change. The AIEC called a demonstration at Mt Taylor to protest at a Gulf Oil uranium mine and mill complex which plans to produce 100 million pounds of uranium. On the same day the Energy Association of Taxpayers, an organisation funded by the energy companies, sponsored an Energy Fair in nearby Grants, the self proclaimed "Energy Capital of the World." Following

a parade with floats carrying the slogans "Who Sabataged Three Mile Island?" and "Uranium for Our Children", New Mexico Senator Harrison Schmidt told the rally:

"I will do everything in my legislative

power to eliminate the need for environmental impact statements for future uranium mines and mills." □

And in the meantime, in the UK, the Central Electricity Generating Board made a £150 million bid for a share in the Ranger

uranium project on native lands in Northern Territory in Australia. According to the *Guardian*, the CEGB bid is to safeguard Britain's growing need for uranium which is expected to increase by 2½ times in the next 10 years. □

Spanish scientists protest over looming cash crisis

Fifteen Spanish scientists last month published an open letter to their government. They claimed that research would be brought to a halt in 1980 if the government did not act quickly to provide funds. The letter, which appears below, was communicated to us by a Barcelona scientist, Dr Juan Subirana, whose further comments appear this week in correspondence (p 424). The scientists' difficulties appear against an annual inflation rate of 25%, an extensive attempt to curb government expenditure, and a near-complete lack of scientific representation in parliament

State-financed research groups in Spain are in great difficulties for lack of money — and if the government fails to take immediate action, their problems will be dramatically worse next year. On 31 December this year financial support for all projects approved by the Comision Asesora de Investigacion Cientifica y Tecnica will end. The projects were announced in 1975 and 1976.

As you know, scientific and technical research in this country has three financial sources:

- Institutional finance through the universities, the council for scientific research (CSIC), and the research centres of ministries of agriculture, industry, health, public works, transport, and communications;
- Grants from Spanish or foreign foundations and those derived from bilateral international agreements; and
- The Comision Asesora de Investigacion Cientifica y Tecnica (CAICT).

The first of these sources, institutional finance, covers little more than maintenance, particularly in the case of the universities and the CSIC. Research funds come mostly from foundation and international grants and the CAICT. In the universities and the research centres of the CSIC at least 80% of the funds available for research come from the CAICT. Yet up to this date there has been no invitation from the Comision for proposals for new projects beyond the end of this year.

Since there are no substantial alternative sources, research — at least in the universities and the CSIC — will virtually cease on 1 January 1980.

The consequences are obvious: projects

underway will have to be abandoned, in many cases with the loss of everything already achieved; and scientists in training and contracted auxiliary personnel will have to leave their laboratories.

The Comision Asesora was established in 1958. Recently it has come to belong to the Ministry of Universities and Research, with its annual budget of 1.1 billion pesetas (\$17 million) dedicated to research. We understand that the Comision is being reorganised, but we do not understand why this reorganisation has halted the existing mechanisms for distributing Comision funds. We also understand that the government has prepared a three-year plan for research support which will be discussed in parliament, but it seems clear that no funds will be forthcoming from that plan until the second half of next year.

For the survival of science, we ask the government, through the Ministry of Universities and Research, to take urgent measures. We scientists signing below believe we speak as representatives of many others when we propose that the government should:

- Immediately announce the offer of Comision Asesora research grants for 1980, and assure that the selection procedure is complete by the end of the year

- As a matter of urgency, put the three-year plan for research before parliament.

So our country may have a minimum of independence and intellectual respect, political changes must go along with deep changes in our scientific and technological policy; but these changes must not be made at the expense of a temporary paralysation of Spanish research.

The day after this letter appeared in the Spanish press, a meeting of the joint council of the CSIC, the universities, and the Ministry of Universities and Research took place. The meeting had already been planned as part of the efforts to create a research policy for Spain. The press commented on 28 September:

El Pais (Madrid)

"The Ministry of Universities and Research is studying the reorganisation of the CSIC as one of the first steps in the redefinition of research supported by the administration. Among other measures there are the preparation of a general report on research in Spain, the three-year (1980-82) plan of research, cooperation with other administrative bodies, etc. In particular the remodelling of the Comision



King Juan Carlos: trouble with science

Asesora de Investigacion Cientifica y Tecnica is also being considered.

"Publication appears imminent in the *Boletin Oficial del Estado* (the official government journal) of a decree that will approve the new bye-laws of the Comision, which is the advisory board of the government in scientific policy, and which has as a main mission the approval of research projects under government support and the distribution of funds to finance them."

La Vanguardia (Barcelona)

"[The Comision Asesora has decided] not publicise now an annual announcement of projects, as requested by a group of scientists in a recent open letter to the Ministry of Universities and Research. The reason is that the 250 million pesetas (\$4 million) available to the Comision would not solve the scientists' economic problems, while it seems more convenient to transfer this quantity to the next year and not to proceed with the announcement before January 1980."

It thus appears that the situation of science in Spain is far from clear, and is continuing to give scientists considerable anxiety. □

Signed: Manuel Ballester, CSIC Barcelona, Nicolas Cabrera, Universidad Autonoma Madrid, Antonio Cordoba, Universidad Complutense, Jose Maria Fontbote, Universidad de Barcelona, Alberto Galindo, Universidad Complutense Madrid, Antonio Garcia Bellido, CSIC Madrid, Antonio Garcia Olmendra, Universidad Politecnica Madrid, Antonio Gonzalez Gonzalez, Universidad de la Laguna, Jose Antonio Jimenez Salas, Universidad Politecnica Madrid, Jose Maria Jover, Universidad Complutense Madrid, Ramon Margalef, Universidad de Barcelona, Francisco Rodriguez Adrados, Universidad Complutense Madrid, Jose Maria Serratos, CSIC Madrid, Alberto Sols, Universidad Autonoma Madrid, Agustin Udias, Universidad Complutense Madrid.

West Germany: a nuclear incident every three days

OVER the past two years, one incident or accident has occurred on average every three days in West German nuclear power stations, according to a recent report of the Bundesverband Bürgerinitiativen Umweltschutz e.v. (BBU), the chief organisation of German environment protection groups and associations. The report, "Accidents in German Nuclear Power Stations. Publication of Confidential Incident Reports of the Federal Government", also claims that most of these incidents have been kept secret. Information for the report was obtained from the files of the Gesellschaft für Reaktorsicherheit (GRS), the German Reactor Safety Society.

Publication of the BBU report came shortly after the German Minister for Research and Technology, Volker Hauff, released part A of the report of a risk study of nuclear power stations in West Germany — the German version of the Rasmussen Report. The study concluded that the probability of an accident is extremely low. Ironically, the author of the study is the GRS, the very same society from which the BBU obtained its information.

GRS is a limited liability company based in Cologne with the Federal government, Bavaria, Northrhine-Westphalia, some technical supervisory bodies and the German Lloyd (an insurance company) as shareholders. In 1974/75, the Federal Ministry of the Interior commissioned the GRS to collect and categorise all data relevant to incidents and accidents in German nuclear power stations (and also, as far as possible, in foreign ones). The aim was to improve safety by mutual exchange of information between utilities and concerned authorities.

In fulfilling this commission, the GRS obtains short standardised reports from the utilities which have been prepared whenever deviations from routine operations have occurred. The deviations or incidents (*besondere Vorkommnisse*) are divided into three categories according to their significance for the safety of the plant and the public:

- Category A includes incidents that immediately threaten safety, for example: emission of more than the maximum permissible amount of radioactivity; breakdown within the primary circuit combined with emission of radioactivity or with coolant leakage.

- Category B includes incidents that are a potential threat to safety, for example: unplanned emission of radioactivity below permitted amounts; breakdown of system components with importance for safety during tests (if not during normal operations).

- Category C includes any other kind of deviation from specified norms and safety regulations.

The utilities themselves categorise

individual incidents so that the GRS simply list the collected data and sends both the incident lists and the individual incident reports four times a year to the Ministry of the Interior, and its other shareholders.

It was these lists and reports from 1965 to autumn 1977, that the BBU has published together with a few comments, interpretations and supplements from other sources. Its report is not easy for the uninitiated to understand, because no systematic interpretation has been done, incidents of differing relevance being catalogued only by time and location. But it is a unique source of information in West Germany. In the introduction, the BBU compares the official risk study report with the confidential lists and incident reports. It concludes that the public has been misinformed on the number of incidents which have occurred. For example in 1976 according to GRS data 139 incidents, 24 in category A, 85 in category B, and 30 in category C occurred. A Federal Government document, however, called "On the peaceful use of nuclear energy" mentions only 14 incidents in 1976, six in category A, six in category B, and two in category C. The BBU also concludes that the public has been misinformed on the "quality" (degree of danger) of certain incidents. It compares the text of a few incident reports (written by the utilities concerned) with assessments of the same incident by other authorities (e.g. technical supervisory authorities). According to BBU a few of the incidents during 1965-1977 came fairly close to a "GAU" (greatest accident to be expected).

The BBU report calls on the Ministry of the Interior to publish all incident reports from autumn 1977 to now, and to provide quarterly, unabridged publications in future. Only then it says, can a rational public discussion take place. It also says that the number of incidents and their type prove that nuclear energy is not safe and it calls for a cessation of operation at all nuclear power stations.

Confronted with these reproaches and

demands, the Federal Ministry of the Interior reacted almost too calmly. It said that the Bund and Länder have been informing the public of all relevant incidents in German nuclear power stations since 1966 and that Parliament is continually informed of all incidents (not only those with safety relevance). Public information is given only on incidents that threaten the safety of a power station and/or the public according to "expert assessment". The 14 incidents, mentioned in the Federal Government's documentation for 1976 are according to the ministry, "representative" and not the total number of incidents.

According to competent experts within the Federal Ministry of the Interior, there is confusion over nuclear information in the FRG revealing a lack of competence and a lack of political awareness that the public must be totally and regularly informed. There is the unique situation in the FRG, that the utilities possess a kind of "copyright" over incidents. This stems from the "Gewerbeordnung" (trade and manufacturing ordinance) a law made for small manufacturers and the "Atomgesetz" (law on nuclear energy). Both are influenced by the same ideas on 'rights of possession'. There is, therefore, no absolute compulsion on the Bund or Länder to publish information on incidents, except where it serves to improve safety conditions (as stated in the "Atomgesetz").

The prime responsibility for nuclear incidents, however, rests with the Länder. The Federal Government, represented by the Ministry of the Interior has only certain supervisory rights over the relevant Länder ministries although it has to inform the Bundestag. Now the Länder provide information on each incident. This used to be done by the Federal Ministry of the Interior before 1976/77, when a first list of incidents (compiled originally for the competent committee of the Bundestag) was published in the ministry's journal *Umwelt* (environment). The list, however, was not complete and the individual incident reports were not published — possibly because of the utilities' "copyrights". As was explicitly said, the list was only "representative".

There now exists an extension to the list for 1977 and 1978. It went to the competent committee of the Bundestag at the end of May 1979 and will be published in *Umwelt* on 12 October. Is this chance or a consequence of the BBU publication? A spokesman for the ministry claimed that "publication was planned long ago, it had to be prepared before the summer recess". The ministry now says that there are no intentions of publishing quarterly or regular lists of incidents or of publishing the individual incident reports made by the utilities.

Klaus Höpfner



Germany to decentralise nuclear waste treatment

GERMANY'S ambitious plans to build a huge nuclear waste disposal centre at Gorleben in Lower Saxony have finally been dropped, despite pressure from the federal government. On 30 September Chancellor Helmut Schmidt and the Minister-President of the eleven German Länder agreed to abandon the plan after a series of lengthy negotiations. And the nuclear industry have now decided to approach the problem in smaller stages.

After an international six-day hearing last May (24 May, page 283), Minister-President Ernst Albrecht of Lower Saxony (CDU) had rejected plans to build an integrated nuclear waste disposal centre at Gorleben. However the Federal Government under Chancellor Schmidt (SPD) continued to adhere to the concept of an integrated waste disposal centre. The result was stalemate and additional delays to nuclear expansion in West Germany — because of the law-courts' insistence on feasible nuclear waste management before granting licences to new nuclear power plants.

But now Chancellor Schmidt and the Minister-Presidents of the Länder have agreed on the following points:

- West Germany is to manage the whole of its nuclear fuel cycle but in small units at different sites.

- No reprocessing plant of any kind is to be built at Gorleben in the foreseeable future. Instead, the possibility of building one or more small reprocessing plants at other sites in West Germany is to be investigated and the final decision on reprocessing is to be taken by the mid 1980s.

- Testing of Gorleben salt domes for final storage will continue.

- Lower Saxony and Northrhine-Westphalia have agreed to build two or three intermediate storage facilities which will be needed for about 20 years before a final solution to the storage problem is developed. The Bavarian criticism that "compact storage facilities" within nuclear power stations "contribute to the intermediate storage problem" has been taken into account.

- Research into alternatives to reprocessing, in particular final storage of unprocessed spent fuel, is to be supported.

But there many unknowns in this political solution. One certainty, however, is that no reprocessing plant will ever be

built at Gorleben — Minister-President Ernst Albrecht is trying to avoid endangering his political career, especially as he is a potential candidate for the chancellorship in 1984 or 1988. A complete new reprocessing plant may never be built especially if ideas to enlarge the existing experimental plant at the nuclear research centre in Karlsruhe are adopted.

However the problems of siting intermediate storage facilities might be only marginally easier than those of siting reprocessing plants and final storage facilities. In Ahaus (Northrhine-Westphalia), for example, which has already been selected as the site for intermediate storage ponds, the CDU lost 20% of their former votes during local elections last week while a recently-formed opposition group won 25% of the votes.

Further, the question of whether the law-courts will accept the intermediate storage compromise as a solution to the waste management problem for the time being is still open. And last but not least, the congress of the governing SPD (Social Democratic Party) will be held in Berlin in December. Nuclear power will top the agenda.

Klaus Hopfner

Soviet psychiatrist attacks 'Snezhnevskism'

MOSCOW'S psychiatric establishment has now produced its own internal dissent — according to US psychiatrist Walter Reich. He was referring to an article, soberly titled "Differentiating Exogenous Psychiatric Illness from Schizophrenia", which was apparently sent abroad by unofficial channels and appeared in the August issue of the US "Archives of General Psychiatry".

Of itself, the article appears to be simply a criticism of current Soviet diagnostic procedures. On the basis of an analysis of more than 300 patients, the author, Dr Eteli Filipovich Kazanets of Moscow's Servskii Institute of Forensic Psychiatry speaks of many "over-diagnoses", "incorrect diagnoses", the necessity for "revising many long-standing diagnoses" and the "criteria, associated with the Snezhnevskii school" which "over-extend the diagnosis of schizophrenia".

For Andrei Snezhnevskii, head of the Institute of Psychiatry of the Soviet Academy of Medical Sciences, all mental illness is some form of schizophrenia. Indeed, he has identified a number of types which seem of specifically Soviet provenance, including "sluggish" schizophrenia which, it appears, has no presenting symptoms except political dissent.

Dr Kazanets, it must be stressed, does not in any way refer to the "political" application of Snezhnevskii's theory — although coming from the Serbskii Institute which has been responsible for

many diagnostic reports on dissidents. Indeed, he does not even say that the patients whom he reviewed were in fact in good mental health — only that they were suffering from brief "exogenous psychoses" which could have been cleared up in a short time, without for the long "rehabilitation" process with compulsory attendance at psychiatric outpatient clinics and domiciliary visits.

Where, then, lies the "daring" which Dr Reich — known as a moderate and cautious commentator — finds in the article? It should be stressed, perhaps, that even apart from its provenance, the study is, in the words of Jack Weinberg, former president of the American Psychiatric Association, scientifically "significant".

Of recent years Snezhnevskii's theory of schizophrenia has made a number of western psychiatrists wonder how long a meaningful dialogue with their Soviet opposite numbers would be possible. Irrespective of the political misuse to which they could be put, Snezhnevskii's views, it was felt, could impose a Lysenko-type isolation on Soviet psychiatry.

To attack these entrenched ideas, Dr Kazanets was obliged to send his manuscript abroad "privately", bypassing the official postal (and censorship) channels — an action which in itself could have serious consequences. Yet the article is, in itself, controlled in tone. Certainly, as western commentators have been keen to point out, he notes that the "prolonged retention of individuals on psychiatric,

dispensary lists long after their recovery infringes on rights" (not "human rights" as it has been misquoted). He also implies, however, that Soviet society as a whole may suffer: the "illegal inclusion of persons not mentally ill on psychiatric rosters" may entail "criminal elements" being unjustifiably freed from "responsibility for their crimes".

Dr Kazanets has long been considered something of a "loner" and "troublemaker" by his colleagues, and it is not surprising that he should be the first to raise his voice in qualified criticism of Snezhnevskii. Nor is it entirely surprising, that some cracks are occurring in the monolith of Snezhnevskism. Already some "establishment" psychiatrists are known to have associated themselves with the dissident "Working Commission for Monitoring the Use of Psychiatry for Political Purposes" (a body which in spite of official harassments is still producing a regular bimonthly *samizdat* bulletin).

One should not, however, be over-optimistic. So far there is little indication that Dr Kazanets has any great following, although at least one of his colleagues has been prepared, privately, to testify to his intelligence and "stimulating" personality. Such an assessment may well be needed in months to come — for already there are hints that certain Soviet "establishment" psychiatrists are referring to Dr Kazanets's challenge as itself a symptom of — "schizophrenia".

Vera Rich

news in brief

US Senate agrees to circumvent environmental legislation: The US Senate agreed last week to support President Carter's proposals to establish an Energy Mobilisation Board to cut red tape on selected large energy projects. This was despite the objections of environmentalists and state and local groups that the board's powers to speed up the review process could undermine many of the environmental laws that have been passed in recent years, and would trample on the rights of local governments. An intense lobbying effort by Carter and by the oil industry managed to secure the defeat of an amendment from Senator Abraham Ribicoff and Senator Edmund Muskie that would have eliminated these provisions from the Bill setting up the board. The measure now passes to the House of Representatives, which is already considering two versions of the Bill, one of which — passed by the House Commerce Committee three weeks ago — would give the board even stronger powers to ignore what it considered to be unreasonable environmental legislation. Meanwhile, a report prepared by a Senate budget committee and issued last week stated that the President's proposal to reduce oil imports through massive development of synthetic fuel production cannot presently be justified on economic grounds.

Fuelling guided missiles can seriously damage your health, as well as causing a major threat to the environment, according to Chinese military experts. Charging a missile with liquid fuel is a delicate task, involving an accurate determination of the temperature in the fuelling trough. Conventional sampling methods entail drawing off a sample of up to 40 kg. This deteriorates in contact with the air during the measuring process, and is afterwards voided, seeping into the soil and polluting the ground-waters. There is also considerable atmospheric pollution and health hazard, apparently from reaction fumes. To overcome these disadvantages (and to save the state the 1500 yuan spent annually on the wasted fuel), two members of the "fuelling squadron" — platoon leader Wang Enchen and technician Xun Chengzhi — have developed a closed-loop test method which gives a direct readout from a temperature gauge permanently fixed inside the test chamber. In a recent broadcast, Peking radio stressed the prolonged research in metallurgy and the science of refractories needed before an effective system was perfected. But now, said the commentator, this sophisticated system has saved state research and development funds, improved working conditions for the fuelling personnel, and shortened the average fuelling time from ten hours to six (and on one occasion, only 2.5).

US experiments carried by Soviet satellite: An international collaboration on the effects of weightlessness on animal and bacterial growth was launched 26 September in the Soviet Union. The major payload consists of 38 white rats and 60 fertilised Japanese quail eggs shared among experimenters from the participating countries which include the US, France and Czechoslovakia. The launch is similar to two previous Cosmos missions in 1975 and 1977 in which the US also participated. In addition to experiments on embryonic growth in both mammals and birds, studies will be made of changes in animal muscle and bone in an attempt to investigate the reasons for calcium loss in the bones and loss of muscle strength by astronauts and cosmonauts during prolonged space flights. The flight will last three weeks.

West German government outlines biotechnology programme: In response to a formal parliamentary question, the Federal Ministry for Research and Technology (BMFT) has published its appraisal of West Germany's present position in biotechnology. West Germany is in the middle rank for biotechnological research, well behind the US and Japan but with a research effort comparable to the UK and France, says the BMFT. In certain sectors, West

Germany has reached international standards — for example in the production of single cell proteins from methanol, in cell culture technology, in basic research for bioreactor development, and in the production of compounds by enzymatic conversion.

For the first time the 1980 research budget contains a separate entry for research and development in biotechnology, amounting to 37 million DM. The government's aim is to improve the current standard of research and to facilitate industrial implementation of research results. The government also wants to reach international standards in the following areas: design and construction of bioreactor systems in sewage treatment; biosynthetic processes; fixation of bio catalysts; gene technology and plasmid research; biological nitrogen fixation; and bioenergetics and renewable raw materials.

French government backs down on faulty nuclear start up: A five-day strike by the socialist and communist trade union federations, the CFDT and CGT, has forced the French government to postpone the loading of its Gravelines 1 and Tricastin 1 nuclear reactors. The French electricity authority will convene health and safety committees to investigate the implication of the fissures in the tubular plates of the heat exchanger (*Nature* 27 September) and will make further checks, not expected to take more than three weeks, on the faulty parts. The existence of the cracks up to 6 millimetres deep was made public on 22 September but the government announced its plans to go ahead with the fuelling on 3 October. Although up to 20 reactors are affected, the government argued that the cracks would have to be 60 millimetres deep to constitute a serious hazard. The government's decision provoked a storm of criticism throughout France as political and environmental groups joined the trade unions in protesting the government's decision. Eighteen organisations issued a joint statement on 3 October calling on the government to "refuse this fantastic industrial gamble which could have very heavy consequences." The faults in the heat exchanger plates raise the question whether the plates could actually break. Collapse of the plates means instant and uncontrollable release of water from the primary cooling circuit which would result in a complete loss of coolant.

WHO report criticises current European occupational health programmes: The World Health Organisation has released a stinging critique of routine health examinations for workers exposed to occupational hazards. The report says that European countries have placed too much emphasis on medical examinations and not enough on elimination of industrial hazards. "The fact is sometimes overlooked that the examination of a worker does nothing to eliminate the hazard . . . Technical improvements should be considered first and the goal should be to make examinations unnecessary by eliminating hazards in industries." The study, a survey of industrial health practices in Finland, the German Democratic Republic, Poland, the USSR and the UK, questions the efficacy of periodic health examinations which are undertaken on a "considerable" scale by European governments. Criticising the lack of knowledge of physicians of the physical and mental requirements of specific jobs, the difficulty of predicting the consequences to health of exposure to industrial hazards and false negative results of many clinical and laboratory tests, the report claims that present practices are wasteful and avoid the real problem of prevention. "If the cost and benefits of the examinations are compared with the really effective alternative, the elimination of the hazards, the present practice in many countries will be shown to be uneconomic."

● The second author of last week's article on geological aspects of nuclear waste disposal (4 October, 332-4) should have been D.A. Gray, not D.A. Greenwood.

Containing Recombinant DNA: how to reduce the risk of escape

David R Lincoln, Laurence R Landis and H Andrew Gray describe a 'fault tree' analysis of containment facilities which, when applied to Fort Detrick (right), enabled them to suggest how to cut the chance of an escape from one in 15 years to one in 22. The authors are from Carnegie-Mellon University, Pittsburgh

The technique of recombining DNA from a foreign source into a host organism could have harmful consequences only after a sequences of events, if at all. An organism must escape from the containment facility, there must be another living system present, the living system must become infected, and the recombinant DNA (RDNA) organisms must bear some harmful action which is released to the living system. (This very generalised framework does not account specifically for differences in the action of toxic compounds, immune responses, or other possible responses.)

Several biological studies have been performed during the last six years which have influenced the understanding of the probability of infecting a living system, and of the infection having harmful effects.¹⁻⁵ However, limited research⁶⁻⁸ has been performed to increase the knowledge of the adequacy of capabilities of the different physical containment facilities for RDNA experiments, or for experiments with known pathogenic organisms and viruses. We describe a framework for assessing the containment system reliability with fault tree analysis.

Engineers use fault tree analysis to identify the various combinations and sequences of component failures which lead to an undesirable event in a system — such as the melt down of a nuclear reactor. The technique is particularly suited to the analysis of complex systems because it can effectively model a vast number of system components.

The procedure is performed stepwise, with the first decision being a choice of the 'top' event, which is an undesirable event associated with the system. The next step is to determine all the necessary conditions, or secondary events, which will cause this top event. If two secondary events must both occur to cause the top event, these two events will be linked in the tree to the top event by an 'and' gate. However, if either of the events will cause the top event, the secondary events will be linked to the top event by an 'or' gate. An ordering of causative events will be generated through subsequent steps, until finally a set of primal events is reached. These are events whose causes need not or cannot be determined.

'Cut sets', which are independent paths or sets of primal events which cause the top event, are then determined. Any cut set having no subsets identical to any other

cut set is defined as a 'minimal' cut set. The frequencies and probabilities of the minimal cut sets are calculated, and the frequency of the top event is the sum of the frequencies of all the minimal cut sets. A more detailed discussion of the procedure and method of calculation has been published previously.⁹⁻¹⁰

Our study focused on one P4 containment facility, Building 550 of the Frederick Cancer Research Centre (FCRC) at Fort Detrick, Frederick, Maryland. A P4 laboratory is the most stringent containment facility for DNA research. In particular, this study examined the escape routes from Rooms 214 and 215, which contain the Class III cabinet areas of the P4 facility. The facility is undergoing some structural changes, and we obtained detailed architectural drawings of the reconstructed building with the cooperation of the engineering staff at FCRC. The drawings included floor plans, ventilation and piping diagrams, and details of the electrical systems.

The FCRC 'Safety and operations Manual' and FCRC personnel themselves, verified procedures for entering an exiting the facility, experiments, and other operations of the facility. Fig.1 shows a schematic which was developed from the architectural drawings of the air ventilation and liquid modes of transport of materials from Rooms 214 and 215.

Air is supplied to Rooms 214 and 215 from 'clean' areas of the building by fans, creating a pressure differential between these two areas and producing a directional air flow. Supply air is passed through two



filters, one low efficiency filter for large particles and one 95% efficient filter. An air pressure difference is also maintained between the room air and the Class III cabinets. Air is exhausted from the rooms and the cabinets through the filters indicated in the diagram.

The sole water inlet to Rooms 214 and 215 is into the Class III cabinets. Before the water enters the facility, it passes through a back flow preventer. No drinking water is available in the rooms, and building drinking water is supplied by a separate line which is tapped off the main line before the water reaches the back flow preventer.

There is one floor drain in each room and several cabinet water drains. Each drain has a minimum six-inch trap and all liquid effluents drain to a common pipe. The liquids are treated by the Fort Detrick Sewage Sterilization Plant, and then by a municipal sewage treatment plant having primary and secondary treatment.

Two additional modes of organism escape were considered after an examination of the facility: contact modes, and natural catastrophes. The contact mode included the human contacts through ingestion, injection into the blood, or transport on skin or clothes, as well as organism contact and escape on any

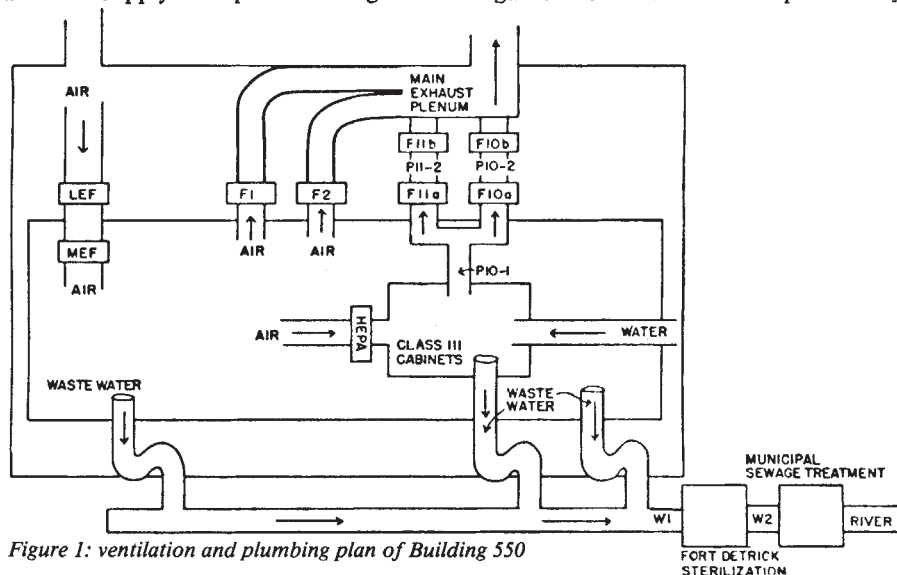


Figure 1: ventilation and plumbing plan of Building 550

materials (instruments, glassware, paper, laundry, media, cultures, etc.) which are transported from the facility.

Certain natural catastrophes could occur during the lifetime of the facility which could reduce or eliminate the effectiveness of all containment carriers. These could include earthquakes, major storms and fires. Although the probability of these events may be remote, the probability of escape given the events is very high. Therefore, these escape routes were also considered.

The top event in this fault tree was considered to be "appreciable number of RDNA organisms escape" from the P4 facility. It is recognised that different numbers of organisms would be used in different experimental protocols. In addition, aerosol generation would be likely to be a function of the experimenters' capabilities.^{6,7} The survivability of organisms in different media (air, water, solid wastes) would also play a role in determining the likelihood of escape of a given number of organisms. The transit time to the exterior of the facility was considered to be very low with respect to organism survival time.⁷ These factors were not considered explicitly in this first application of fault tree analysis to recombinant DNA containment facilities. It should be noted however, that more detailed studies to develop appropriate models and experimental data could lead to an incorporation of these variables in a second generation tree.

The four major escape routes are shown in the fault tree in Figure 2:

- air ventilation transport,

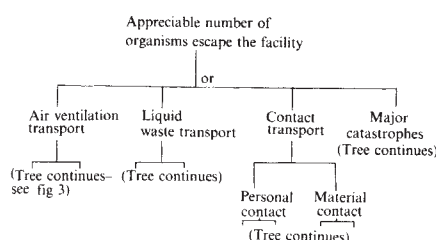


Figure 2: fault tree showing major escape routes

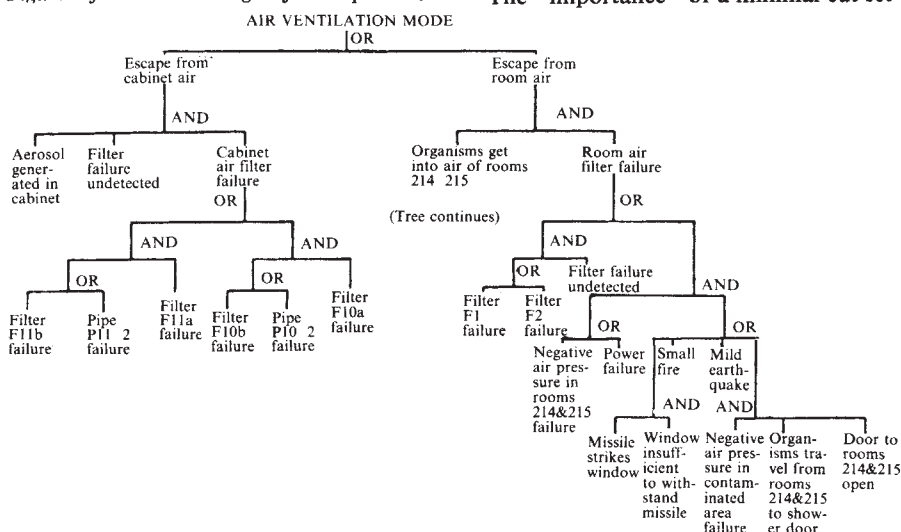


Figure 3: part of fault tree showing risks through air ventilation

- liquid waste transport,
- contact transport, and
- major catastrophes.

The events leading to air ventilation transport are shown in Fig 3. The primal events (57 in the whole tree) can be found at the bottom of the tree. Details of the complete tree are available from the authors.)

For each primal event there is an associated frequency of occurrence, λ , which is the inverse of the mean time interval, in which the event will occur exactly once. The mean time to repair, τ , in the context of fault tree analysis, is the time needed either to repair the fault or to move the system to a safe state. For some primal events, it is easier to assign an unavailability, q , instead of λ , and τ . Unavailability is the probability that a component is not available.

The components of the P4 laboratory have been operational since April 1977, which was an insufficient time period for developing a useful data base of operations. However, similar component designs exist in other industrial applications, and estimates for q , λ and τ were developed after a discussion of the primal events in the fault tree with G.J. Powers, Department of Chemical Engineering, Carnegie-Mellon University. A list of the values used in the base case fault tree is available from the authors. It should be recognised that these values can be updated with experimental data as they become available.

The minimal cut sets were developed from the fault tree by applying the algorithm developed by Powers¹⁰, revealing over 200 potential escape routes. The frequency of each cut set was calculated using our estimates of q , λ and τ with the frequency equations of Powers.¹⁰ In order to obtain the frequency of the top event, the frequencies of the minimal cut sets were summed. The mean frequency of the top event 'escape of an appreciable number of organisms', was calculated to be once in 15 years.

The "importance" of a minimal cut set

Table 1: CUT SET IMPORTANCE	
Cut Set	Importance to Escape Frequency

Organism injected into blood	0.383
Filter F11a and F11b failure;	
filter failure undetected	0.191
Transport container leaks	0.077
Organism inhaled in Rooms	
214 and 215; pipe P10-1 failure	0.046
Filter F10a failure; pipe	
P10-2 failure; filter failure	
undetected	0.025
Filter F11a failure; pipe	
P11-2 failure; filter failure	
undetected	0.025
Appreciable number of	
organisms in building;	
major storm	0.023

Table 2: PRIMAL EVENT IMPORTANCE

Event	Importance to Escape Frequency
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in a fault tree is defined as the frequency of that minimal cut set divided by the frequency of the top event.¹⁰ This may be interpreted as the probability that the events of a minimal cut set have occurred, given that the top event has occurred. From Table 1, it can be seen that approximately 75% of the escape potential can be attributed to three minimal cut sets. With the top eight cut sets, approximately 95% of the escape potential is accounted for.

The "importance" of a primal event is defined as the sum of importances of every minimal cut set in which the primal event is an element.¹⁰ The potential "weak" links in the containment system are further identified in Table 2. Of the top six primal events in importance, five involve the high efficiency particulate air (HEPA) filters F10a, F10b, F11a, F11b, which are located in the exhaust stream of the Class III cabinets. In addition, the event of injecting the organisms into the experimenter's blood by accident is also a significant contributor to the potential for organism release.

The frequency of escape can be modified by changing system and procedural variables. We tried modifications of the parameters of all primal events that appeared in both the set and primal event importance tables (tables 1 and 2). In particular, it was found that changes in the HEPA filter parameters will significantly modify the escape frequency.

In the base case, the mean escape

The authors would like to acknowledge the cooperation of Drs W Emmett Barkley and John Nutter, National Institutes of Health, Washington, DC and the assistance of Orley Bourhard, Everett Hanel, Stanley Nagel and Irv Swope at the Frederick Cancer Research Center.

frequency is once in 15 years, assuming a mean HEPA filter failure rate once every four years. However, if this is decreased to once in eight years, the mean escape frequency decreases to once in 22 years. On the other hand, if the filter failure rate increases to once in two years, the mean escape frequency increases to once in seven years. If the filter mean time to failure detection is reduced from six months to 1.5 months, then the mean escape frequency again declines to once in 22 years. The impacts of other system modifications can be assessed in a similar manner.

We recognise that our fault tree — part of which appears in figures 3 and 4 — does not describe all the system parameters and modifications which may be of interest in a more complete description of escape likelihood from the P4 system. In particular, there remain considerations of:

- a more explicit description of the dependency of the likelihood of escape on the amount of released material, possible concerns that the organism survivability, and therefore potential for harmful impact, may depend on the mode of escape,
- the effect of alternative experimental protocols on the escape risk, and
- inclusion of experimental data on λ , τ , and q for system components. In addition, further examination of the fault tree for common mode failures would be useful. It is intended that future studies will reduce some of these presently perceived deficiencies.

This study does not describe whether our calculated escape frequencies pose a significant risk. The probability of a harmful consequence is the product of several probabilities involving biological details of the recombinant organisms and their escape probability. Further studies would be needed in order to determine whether or not a P4 laboratory provides an "acceptable" risk. □

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Full steam ahead to save Kenya's fuel

HARD-pressed by the rising price of oil and a growing shortage of fuelwood, Kenya, like many developing countries, badly needs alternative sources of energy. Fortunately, it has at least one: a huge natural reservoir of boiling hot water some 1,000 metres beneath the Rift Valley about 80 kilometres from Nairobi.

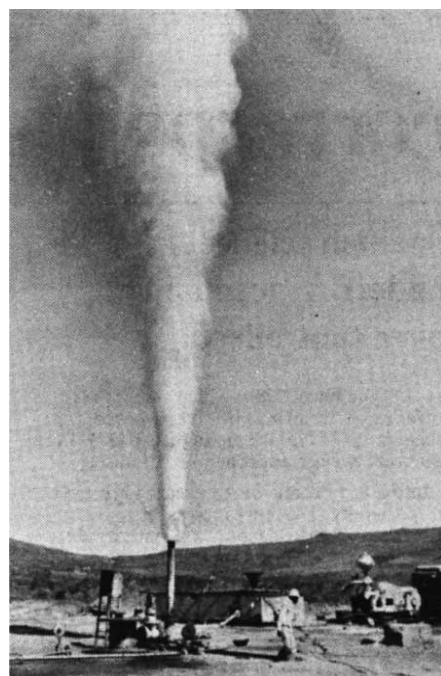
Interest in the area as a possible site for a geothermal power station began in the 1950s, but detailed exploration (financed by the United Nations Development Programme) did not begin until 1970 when four wells were drilled in a wild, semi-arid area known as Olkaria, about five kilometres south of Lake Naivasha. Eleven wells have now been drilled there, and a contract was recently placed for construction of the first 15 MW power station which will feed into the national grid by mid-1981.

The Olkaria Power Station will be the first full-scale geothermal power station in Africa (a 220-KW plant was installed in Zaire at a metal mine in the 1950s, but was costly and inefficient and was closed down with the mine). When Olkaria goes into operation, it is expected to supply about 5% of Kenya's electrical power. Another 15MW generator is planned for operation in late 1982, and the station's capacity will be expanded to reach 90 MW in the next 10 years. Total energy available from the reservoir has been estimated at 170 MW for 25 years.

Kenya has no known fossil fuel resources, and the rising cost of imported oil recently prompted the President, Daniel Arap Moi, to call for reduced consumption. But as T.S. Tuschak, a Canadian energy consultant currently on loan to the Kenya government through the UNDP, pointed out recently (July 5, page 2) savings in the essential sectors can at best be 7%, and consumption by private transport can only be reduced by about 10%.

Kenya's economy is divided into two sectors: the industrialised and the rural. The industrialised sector gets about 85% of its total energy supply from oil, the rest from hydroelectricity. The rural economy's energy source is almost 100% wood or charcoal. And while Kenya has not suffered as much as some countries from deforestation, a fuelwood shortage is likely and has already occurred in some areas. Population is increasing at 3.9% a year, causing not only increased wood consumption but declining land availability. Fuelwood consumption is currently 20 million tons annually.

The government's forestry department has introduced conservation and reforestation programmes and has shown some interest in agroforestry, and



Rift Valley gushers, courtesy of the Kenya Power Company

public attention has been drawn by various groups to the advantages of tree-planting. But it is not possible to wean the mass of the population away from its reliance on wood as its principal source of energy.

Kenya's unused hydro-electric power potential is 600-700 MW. Total electricity demand, which has grown at 8-9% per annum and is expected to continue at that rate for the balance of the century, should reach 1200 MW by AD 2000. This would call for 1500-1600 MW installed capacity. Development of all the HEP potential together with the present installed capacity of about 400 MW would leave a gap of 400-500 MW. The total geothermal resource is believed to be between 170-500 MW which could fill that gap.

Kenya has so far evolved no rural energy policy. While there are arguments for and against rural electrification schemes in developing countries, Mr Tuschak believes that an increase in electrical consumption in certain areas of Kenya could reduce its growing consumption of wood.

"In my estimation there are about 1-1.5 million people in the rural sector who are better off in financial terms and can afford to pay for commercial energy, or at least part of the cost," he told *Nature*. "Many of these people have already requested electricity and others would be happy to have it. If they are supplied with electricity they will stop consuming wood, and Kenya could perhaps cut down wood consumption by 10% or more".

Although hydro stations provide most of Kenya's electrical energy at present, hydro generation is expensive to run because the country's rivers have highly variable seasonal flow. Capital costs are also high. Under such conditions, geothermal plants offer an economic supplement.

David Spurgeon

correspondence

Spanish science: underfinanced and over centralised

SIR, — The uncertainty which research is suffering in Spain was briefly reviewed in a recent issue of *Nature* (9 August, p438). In the meantime we are approaching collapse.

In the last decade most research groups were supported by three- or four-year grants administered by the Comisión Asesora de Investigación Científica y Técnica. These grants are essential to cover running expenses as well as contracts for young scientists waiting for a stable position. The last distribution of grants took place in 1977 and all grants presently in existence terminate in December 1979. No new distribution scheme has been publicised, so that up to this moment we do not know the 1980 situation.

Even if a distribution of grants is announced in the next few weeks, processing them will take several months. Under these circumstances many scientists are forced to leave the country. Only emergency action will stop irreversible damage to research in Spain: namely, to extend automatically current grants for one year more.

The Ministry of Universities and Research is certainly preparing a three year plan (1980-1982) for the development of research in Spain. We are all eagerly waiting for its guidelines, but time is running short.

Another stumbling block in the development of research in Spain is its continuing centralism. A clear example is the Spanish Research Council (CSIC). Over two thirds of its activities and scientists are located in Madrid, with even a larger fraction of its budget concentrated in Madrid. This fact prevents a rational development of research elsewhere in Spain, close to places (industry, agriculture) where it is most needed.

In 1977, 187 new positions for scientists were provided; 70.6% of them were awarded to Institutes in Madrid. Presently another 198 new permanent positions are in the process of being provided; again over 60% of them will be for Institutes in Madrid. These numbers are not trivial, since the new positions mentioned represent 28% of the total permanent positions of the Spanish Research Council.

A peculiar geography of science has arisen through the years: there is not a single research institute in the Basque country, there are more Catalan biochemists working for the CSIC in Madrid than in Barcelona, there are more Catalan physicists working for the French CNRS than for the Spanish CSIC . . . This trend needs urgent modification. Drastic preference for new positions should be given to new and existing groups located in other areas of Spain.

At present a lack of funds and guidelines, together with the maintenance of centralism are producing irreparable damage to the development of research in Spain. We very much hope that the announced three year plan may open a brighter future.

Yours faithfully,

JUAN A SUBIRANA

*Escuela Técnica Superior
de Ingenieros Industriales, Barcelona*

Egyptian science

SIR, — I must comment on the article by Ziauddin Sardar (2 August, page 350).

"Divided and uncertain" does not reflect the reality of the Egyptian scientific community. As a scientist, and science editor for *Al Ahram* for the last twenty years, I will try to be objective, realistic and frank. The Egyptian scientific community, the biggest in the Arab world and Africa by any measure, is not divided regarding scientific cooperation with Israel. Instead the majority are for international scientific cooperation which they are practising all over the world in spite of shortcomings, drawbacks and loopholes in Egypt; and regardless of the sufferings of scientists and lack of stability and funds due to continuous wars which have drained and exhausted our already depleted resources. Cooperation with Israel, on what basis?

In the meantime the powerful Israeli scientific community is silent and negative regarding the dangers threatening the peace process, mainly the insistence of the Israeli politicians and policy makers on depriving the Palestinians — including the scientific community — from their legitimate human rights to live in peace in their homeland. The Israeli scientific community is meanwhile raising hell against the Soviets for injustice against some individual Jewish scientists.

To be frank, the Egyptian scientific community does not like or agree with the Israeli superiority complex, the assumption that Israel is the only cradle of science and technology in the Middle East, and that it is an oasis of development and civilisation in a desert of backwardness inhabited by ignorant Arabs, including the Egyptians. Not bearing in mind the colossal funds and privileges Israel is getting from the US and other rich Jewish communities, the Israeli scientific community must realise and recognise that Egyptian scientists — inside Egypt and the brain-drained Egyptian scholars in the US, Europe and other Arab countries — are competent despite all the shortcomings. Unless confidence prevails, signed treaties will not help either Israeli or Egyptian scientific communities. We in Egypt refuse the Israeli arrogance in science and technology, including the assumption that Israel will teach the Egyptians and lead them to prosperity. Egyptians are certain (and not uncertain as Mr Sardar says) that comprehensive and just peace must include the Palestinians who have one of the highest rates of education of scientific personnel in the area, and we need their cooperation too, to build peace.

The Egyptian scientific community is certain and undivided about one thing: Egypt will still be part of the Arab world and cannot substitute Israel for Arabs, not only for religious, moral, ethical and historical reasons, but for practical, economic and psychological reasons too. The Arab world is the area where the Egyptian scientific community is practising and will continue to practise its human superiority, and make money too. This cannot be fulfilled in the cooperation with Israel.

Regarding Nechemia Meyer's article "Israel: a one sided love affair", I quite agree with the title but not with the content. The negative response to the willingness of the Israeli scientific community to enter into bilateral relations with Egyptian institutions and the story of the experience of the Israeli scientists in Egypt is very normal. As long as

the Israeli politicians and policy makers still insist on flagrantly depriving the Palestinians of their right to live peacefully in their homeland, and the negative attitude of the powerful Israeli scientific community continues, the chance of Egyptian-Israeli scientific cooperation will be very marginal and meagre. Cooperation cannot be enforced by treaties, especially between scientists. Cooperation can start only when confidence is prevailing — and this is not the case yet. The Egyptian scientific community is part of Egypt, with all its aspirations for a just and comprehensive peace for Egyptians, Israelis and Palestinians.

Yours faithfully,

SALAH GALAL

Cairo

Highlighting persecution

SIR, — Dr Lorch (13 September, page 98) is right to emphasise the large themes of international solidarity. Vera Rich has from time to time highlighted smaller, but not unimportant, instances of persecution of individual scientists. Her facts are sometimes incomplete, but aren't they hard to come by? If Dr Lorch finds it easy, perhaps he would find out why the Fields Medallist Gregory Margoulis was not permitted to attend the Helsinki Congress of Mathematicians. Some of your readers would like to know.

As to the USSR relying on its own scientists, Dr Lorch will find an interesting example of masochistic circumcision if he looks at the USSR reproduction of *J. Chem. Soc. Faraday Transactions II* (69) 1973 page 1104; it deals with the . . . Dagonadze theory. The missing name is Levich.

Yours faithfully,

H. KESTELMAN

University College, London

Decentralising science

SIR, — This area is exactly such as you have in mind in your editorial 'Another sort of brain drain' (13 September). Apart from the excellent Agricultural Department at Aberystwyth 60 miles away, scientific and technological activity here is confined to teaching and the handful of people required to run the three oil refineries and the power station in Milford Haven.

Yet these massive capital intensive plants frequently find their way into lightly populated rural areas of the country, and could serve as a focus for a far more developed scientific community — enjoying incidentally lower establishment cost and infinitely preferable environmental regimes. It may not be fashionable or desirable to adopt the scientific cities of Soviet Siberia: but everyone would benefit from a sensible decentralisation of scientific work — not least from the elimination of time loss and stress inherent in daily travel to virtually any location in South East England.

Yours faithfully,

DAVID GREEN

Haverfordwest, Dyfed, UK.

news and views

Patterns in the abundance of parasites on plants

from Robert M. May

What determines the total number of parasitic species to which a particular species of genus of plants is host? This question—with 'parasite' interpreted broadly to range from herbivorous insects to fungi—has lately attracted much attention.

A primary factor is the geographical range of the host plant, with greater range implying more parasite species. As reviewed by Southwood (*Colloq. Int. du C.N.R.S.* 265, *Comportement des Insectes et Milieu Tropicale*, 471; 1977) and Strong (*A Rev. Entomol.* 24, 89; 1979), various studies have shown that the logarithm of host-species range accounts for some 20–60% of the variation in the number of host-species for a panoply of associations including insects on trees in Hawaii, Britain and Europe, leaf miners of oak in California, gall-forming wasps on oaks in North America, arthropod pests of tea, cacao, and sugarcane, diseases of crops in the continental United States, and parasitic fungi of British trees and North American plants. Three recent papers go beyond this, seeking to understand the causes of the residual variation in 'species richness' of parasites, after such 'species-area' effects are factored out: Strong and Levin (*Amer. Nat.* 114, 1; 1979) analyse an eclectically compiled array of data on the insect associations of 214 genera of British plants and on the fungal parasites of 323 species of US plants; Lawton and Schroder (*Nature* 265, 137; 1977) examine the insect fauna of some 70 species of British herbs and shrubs; and Lawton and Price (*J. Anim. Ecol.* 48, 619; 1979) deal with the narrower group of larval agromyzids that mine the leaves and stems of some 60 plant species in the family Umbelliferae in Britain.

Strong and Levin divide the plants into trees, shrubs and herbs. They show that there is no significant difference among these three categories in the way species richness of parasites increases with geographical range of the host plant, but that trees systematically tend to have more parasitic species than do shrubs, which in turn have more than herbs (per unit area of geographical distribution). More precisely,

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their 31 genera of British trees have an average of 28 species of insects, 40 genera of shrubs average 10 insect species, and 143 genera of herbs average around 5 insect species. The number of species of parasitic fungi average around 15 for their 127 species of North American trees, 7 for 70 shrub species, and 5 for 126 herb species. Lawton and Price classify their umbellifers according to the mean physical size of the plant (the geometric mean of the normal maximum and minimum heights of mature plants, as given in Clapham, Tutin and Warburg's *Flora of the British Isles*) and according to leaf form; these are both measures of the 'architectural complexity' of the plant. They will find that species-area effects account for 32% of the variance in species richness of mining insects, and that plant architecture explains some 20% of the remaining variance.

Within any one of their three growth-form categories, Strong and Levin find that most further comparisons reveal no discernible differences in species richness of parasites. Thus American conifers and flowering trees support roughly the same number of species of parasitic fungi, and grasses do not differ significantly from herbs in general. Likewise, British monocots, grasses, aquatic and bog plants, sedges and composites do not differ discernibly from herbs in general in the number of insects species they support per unit host range. Classifying the host plants by life-history strategy, Lawton and Price were unable to determine any significant difference in the number of agromyzid species associated with annual, biennial or perennial umbellifers; in this respect the umbellifers resemble European Cynarae (Lawton and Schroder, *Proc. IV Int. Symp. Biol. Control of Weeds*, ed. T.H. Freeman, University of Florida Press, 1978). Two comparisons within the herb-shrub-tree categories do, however, show differences; ferns support fewer insect species than do other herb genera in Britain; and weedy herbs support fewer fungal parasites than do climax and subclimax herbs in the US. I shall return to these two points below.

Strong and Levin summarise their results by proposing "that the greater size and morphological complexity of trees than

shrubs, and than herbs in turn, account for these patterns in pest species richness; larger more complex hosts present a greater diversity of pest niches". This matches with Lawton and Schroder and with Lawton and Price's suggestion that plant 'architecture' is a significant component in accounting for patterns in the number of parasitic species, a theme which is further explored in several of the papers in the Special Symposium on Insect Diversity held in 1977 by the Royal Entomological Society (see the chapters by Southwood, Lawton and May in *Diversity of Insect Faunas*, eds Mound & Waloff, Blackwell, 1979).

The finding that ferns support fewer species of parasites than do other herbs can be challenged on the grounds that the fauna of ferns are typically less well studied than those of seed plants. But a meticulous study of the invertebrate fauna of the fern *Pteridium aquilinum*, or bracken, in Britain (Lawton *Bot. J. Linn. Soc.* 73, 187; 1976) suggests that ferns do indeed possess a relatively impoverished fauna of insect pests; adjusted for its range in Britain, bracken does not have a rich pest fauna relative to other herbs, even with Lawton's extremely complete data. Lawton's study encompasses the chemistry of bracken, suggesting it has five distinct modes of chemical protection. It may be that ferns generally possess a greater diversity of biochemical defences than do other herbs, which affords them relative freedom from pests.

The conclusion by Strong and Levin that weedy herbs tend to have fewer fungal parasites than other US herbs is supported by Lawton and Schroder's finding that weeds and other annuals tend to have fewer insect pests than perennial herbs. This tendency is, however, not manifested among Lawton and Price's umbellifers. Moreover, the data for fungal species on weedy herbs is very noisy, in the sense that the species-area regression accounts for only 12% of the variance (in contrast to 30–50% for other herbs, shrubs and trees). The question is further clouded by the fact that the man-made disturbances, which have greatly increased habitat for weeds and spread them so widely throughout the US, have mainly occurred only over the

past 200 years. All this is a pity, as patterns in the relative number of parasitic species associated with weedy herbs could shed light on Feeny's (*Rec. Adv. Phytochem.* 10, 1; 1976) ideas about plant 'apparency'. According to this hypothesis, 'apparent' plant species (which are large, more long-lived, and tend to occur later in succession) are "bound to be found" by herbivores; they therefore need general (quantitative) defences that reduce digestion in herbivores and that cannot easily be breached by the ability of parasites to evolve resistance to plant-produced poisons. Conversely, ephemeral or 'inapparent' plants emphasise reproduction and dispersal, and minimise the metabolic cost of defence against herbivores by use of (qualitative) poisons that are effective in small doses. It is not clear what these ideas imply for the correlation between species richness of parasites and host plant 'apparency'; as Strong and Levin observe, "The null hypothesis that chemical differences do not result in different numbers of parasite species for plant taxa should be tested. One way of doing this might be to look for patterns within residuals about the pest species/host area regression line for each growth form". More work is needed before any generalisation can be made here.

In short, something like 50% in the variance of species richness of parasites among host plants can typically be attributed to the geographical range and architectural complexity of the host. Strong and Levin, and Lawton and Price, advance various suggestions about the other 50%. Lawton and Price favour the idea "that the subtle accumulation of specialists (e.g. miners and gall formers) may continue over very long periods of evolutionary time". This could result in much variability if their 60-odd agromyzid-umbellifer associations were at many different stages along the road to eventual equilibrium. Most of the evidence, however, suggests that the total number of parasite species on host taxa usually reaches an asymptote within short periods of evolutionary time (see for example, Gilbert in *Analysis of Ecological Systems*, eds Horn, Mitchell & Stairs, Ohio University Press, 1979); Strong, *op. cit.* 1979). Other explanations for the residual variability include incomplete knowledge of the parasite fauna, differences in the abundances of plant species of equal geographical range (common plants could well have more species of parasites than rare ones, geographical ranges being equal), and variation in the efficiency of the plant's biochemical defences. On top of all this is the possibility, recently and cogently emphasised by Simberloff and others, that substantial differences arise from historical and evolutionary accidents, and have no discernible deterministic cause.

Stepping away from the details, we can

see these thoughtful and technically sophisticated studies as a paradigm of much of the contemporary search for patterns in ecosystems. Depending on one's cast of mind, one can derive pleasure

from the systematic explanation of roughly half the variability in the number of parasite species on plants, or pain from that half of the variability that remains unexplained. □

Dissecting the red cell membrane skeleton

from Samuel E. Lux

To survive in the circulation red blood cells must be both durable and flexible: durable enough to withstand the turbulent cardiac passage and flexible enough to negotiate the narrow portals of the spleen. These dual demands are the responsibility of a submembranous protein network which functions as a 'membrane skeleton'. Because defects in this structure are probably responsible for a number of important haemolytic anaemias (see Lux *Semin. Hematol.* 16, 21; 1979 for a review) and because it is a model for cytoskeletons of more complex and less accessible cells, a great deal of effort has been expended dissecting and reassembling the skeleton of the human red cell. This analysis is currently proceeding at an exceptional pace and seems, at last, to be leading to a comprehensible picture of skeletal anatomy.

Definition of the membrane skeleton

Operationally, the membrane skeleton is defined as the insoluble residue which remains after extraction of intact red cells or their isolated membranes with the nonionic detergent Triton X-100. Under the electron microscope this structure is an anastomosing network of twisted, irregularly oriented microfilaments dotted with globular protrusions (Hainfeld & Steck *J. supramolec. Struct.* 6, 301; 1977; Liu *et al. Fed. Proc.* 7, 1508; 1978). It comprises about 60% of the membrane protein mass and includes all of the spectrin (bands 1 and 2), actin (band 5), ankyrin (bands 2.1, 2.2, 2.3 and 2.6) and band 4.1; and a portion of the proteins designated bands 3, 4.2, 4.9 and 7 (Fig. 1). Spectrin, actin and bands 4.1 and 4.9 form the core of the structure since the skeleton retains its shape and structure when the other components are eluted with hypertonic KCl (Sheetz *Biochim. biophys. Acta*, in the press) but disintegrates if either spectrin or actin are removed (Sheetz *J. Cell Biol.* 81, 266; 1979).

Spectrin structure

Spectrin, the major skeletal protein (60 to

75% by weight), is composed of two enormous subunits, bands 1 (molecular weight 240,000) and 2 (220,000) which are structurally similar but are not identical (Anderson *J. biol. Chem.* 254, 939; 1979). The protein has been intensively analysed since its discovery by Marchesi and Steers more than a decade ago (*Science* 159, 203; 1968). In the past few years purified spectrin heterodimers (band 1+2) and heterotetramers (band 1+2)₂ have been isolated and sized (Ralston *Biochim. biophys. Acta* 455, 163; 1976; Kam *et al. Biochemistry* 16, 5568; 1977), the equilibrium relationships of these molecules have been defined (Ungewickell & Gratzer *Eur. J. Biochem.* 88, 379; 1978), and the tetramer has been identified as the physiological species. However, because spectrin has unusual hydrodynamic properties and is difficult to see in the electron microscope, two of the most critical pieces of information — the shape and molecular conformation of the molecule — have resisted definition. Fortunately, Shotton, Branton and their colleagues (*J. molec. Biol.* 131, 303; 1979) have recently discovered that spectrin's molecular outlines can be captured in platinum-carbon replicas formed by low-angle shadowing. These molecular casts clearly show that spectrin dimers are long (~1000 Å), slender (~50 Å), worm-like molecules in which the two subunits are aligned in parallel and variably coiled about each other. Individual molecules assume many different conformations and must be quite flexible — a property which may be critical for normal membrane pliancy. Spectrin dimers join end to end to form tetramers, but high order oligomers are not seen. This confirms the work of Ralston and Kam noted above and indicates that the dimers join head to head instead of head to tail, since, in the latter alignment at least some hexamers, octamers, and so on, should be formed. Because spectrin subunits seem to be tightly attached at their extremities, Shotton postulates that complementary sites exist at the ends of each of the sister subunits and suggests that the dimer-tetramer interconversion simply involves a reorientation of the sites at the heads of two neighbouring dimers so that each subunit pairs with its sister chain on

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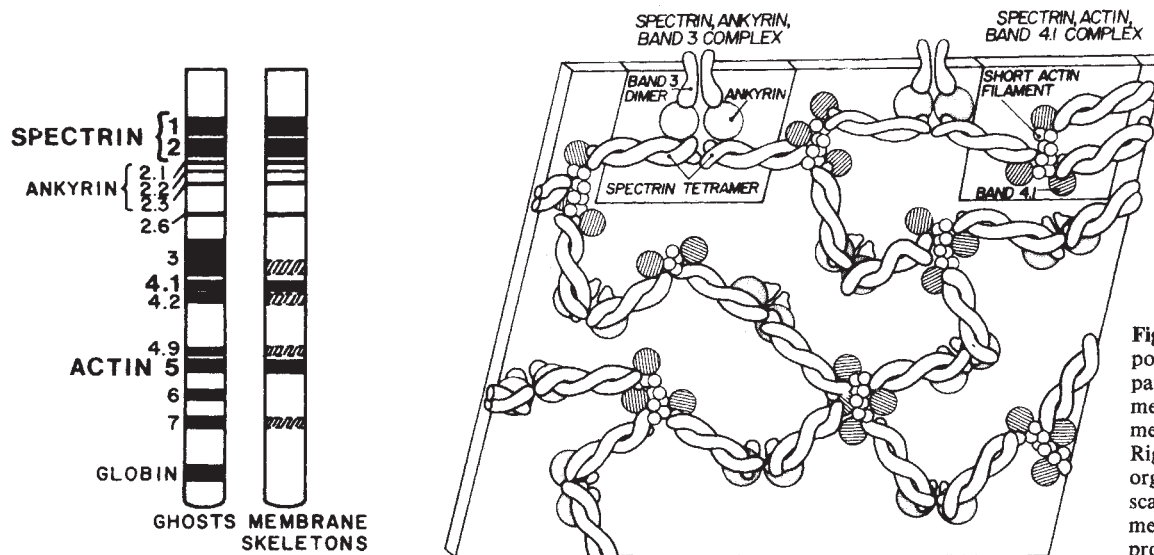


Fig. 1 Left, SDS polyacrylamide gel patterns of red cell membranes (ghosts) and membrane skeletons. Right Postulated organisation (not to scale) of the major membrane skeletal proteins.

the adjacent dimer. This is an attractive hypothesis since the cleavage of existing bonds (the first step in the dimer-tetramer interconversion) could account for the unusually high activation energy of the process (Ungewickell & Gratzner *op. cit.*).

The spectrin-actin-band 4.1 complex

If pure spectrin dimers can only associate to tetramers, then clearly other molecules are required to form the basic skeletal framework. Their identification has been approached in two ways: by dissection and reassembly. Sheetz finds he can strip away all of the skeletal proteins except spectrin, actin and bands 4.1 and 4.9 and still retain an intact skeletal substructure. This suggests that one or more of the latter three proteins provides the critical cross-linking function. Studies reported recently in *Nature* (280, 811; 1979) confirm this postulate. Ungewickell and his colleagues observe that mixtures of purified spectrin tetramer, polymerised actin (F-actin) and band 4.1 combine at physiological ionic strengths into pelletable ternary complexes and, in some cases, gel. No complexes are formed from binary mixtures of these proteins or from ternary mixtures if the actin is unpolymerised (G-actin). Electron micrographs of isolated complexes show F-actin filaments bridged by fine curved fibrils which presumably represent spectrin tetramers. The pictures suggest the actin-binding regions of the tetramer are near the distal (tail) ends of the molecule. Dimeric spectrin attaches to actin filaments but, being monofunctional, is unable to cross-link them.

Independent evidence from several other laboratories largely corroborates this picture. Fowler and Taylor (*J. Cell Biol.* 79, 222a; 1978) have reported that oligomeric complexes of spectrin, actin and band 4.1, extracted from red cell membranes cause actin to gel and that purified spectrin dimers or tetramers lack this activity, suggesting a requirement for band 4.1. More recently (personal communication) they discovered that this

effect can be mimicked simply by adding purified tetramer and band 4.1 to actin solutions. Interestingly, they find the reaction is maximal at physiological concentrations of calcium (10^{-7} – 10^{-8} M), which may explain, at least in part, the deleterious effects of this ion when it accumulates in red cells. At lower ionic strengths, Cohen and his coworkers (personal communication) observe that purified spectrin induces actin gelation in the absence of detectable band 4.1 and Tyler and Branton (*Proc. natn. Acad. Sci. U.S.A.*, in the press) report that purified band 4.1 binds to spectrin in the absence of actin, indicating that stable binary complexes can exist under certain circumstances. Electron micrographs of spectrin-4.1 complexes show that the binding site of band 4.1, like that of actin, is located near the tail of the spectrin dimer.

These studies are all consistent with a skeleton composed of long, flexible, bifunctional, spectrin tetramer strands cross-linked at each end through interactions with actin and band 4.1 (Fig. 1). While this model of the skeleton is greatly advanced compared to earlier renditions, many important questions remain. For example, what is the state of the actin? The experiments of Ungewickell *et al.* and observations of Brenner and Korn (*J. biol. Chem.* 254, 8620; 1979) indicate that actin must be polymerised to complex with spectrin. But actin filaments are not visible in electron micrographs of red cell membranes and the amount of actin present (about four molecules per spectrin tetramer) is not compatible with the existence of long actin filaments. This leads to the conclusion that actin must exist in short filaments or 'protofilaments' (Cohen & Branton *Nature* 279, 163; 1979; Ungewickell *et al. op. cit.*), and, by extension, implies that some mechanism must exist to limit the extension of such filaments. The point is by no means settled however, since there is evidence that oligomeric complexes of spectrin, actin and band 4.1 may contain some G-actin

(Ralston *Biochim. biophys. Acta* 510, 283; 1978). Nevertheless, at present it seems likely that much of the actin in the skeleton is organised as short filaments. If true this leads to other questions. Are all these filaments the same size? Are the number of spectrin tetramers which branch from the filaments constant or variable? In the model shown in Fig. 1, I assume that variable numbers of spectrin molecules are joined by actin filaments of variable length. This hypothesis would explain the disordered organisation of the skeleton seen by electron microscopy, but other explanations are also possible. For example, the disorder could simply be due to the inherent flexibility of the spectrin molecules. Alternatively spectrin may have multiple binding sites for actin and/or band 4.1 near its distal end which are variably filled.

The spectrin-ankyrin-band 3 complex

The interaction of the red cell skeleton with the overlying lipid bilayer is better understood than the organisation of the skeleton itself, thanks largely to a series of elegant studies which originated in the laboratory of Daniel Branton at Harvard. The story began with the observation that the red cell contains about 100,000, high affinity ($K_D = 10^{-7}$ to 10^{-8} M) binding sites for spectrin on its inner surface (Bennett & Branton *J. biol. Chem.* 252, 2753; 1977). Subsequent chapters revealed that each of the sites resides in a 72,000 molecular weight chymotryptic fragment (Bennet *J. biol. Chem.* 253, 2292; 1978), that antibodies to this fragment label only bands 2.1 and 2.2 (Bennet & Stenbuck *J. biol. Chem.* 254, 2533; 1979), and that peptide maps of these two bands and bands 2.3 and 2.6 are homologous with the 72,000 molecular weight piece (Luna *et al. J. biol. Chem.* 254, 2526; 1979; Yu & Goodman *Proc. natn. Acad. Sci. U.S.A.* 76, 2340; 1979). This suggests that the lower molecular weight piece (Luna *et al. J. biol. products of the parent band 2.1 molecule. At present it is uncertain whether they are*

formed in intact red cells or are an artefact of membrane preparation.

Band 2.1 and its progeny have been variously named ankyrin (Greek, anchor), nectin (Latin, to connect), and syndein (Greek, to link together). Ankyrin was chosen first, but because this name had already been given to a plant protein and because it soon became clear that ankyrin was not the true anchor (see below), the protein was rechristened nectin. Unfortunately this name was also a duplicate, so a second change to syndein was made. Some of those working in the field continue to use ankyrin while others have adopted the revised terminology. Presumably the rest of us will have to choose between these appellations (I prefer ankyrin) or make up our own, at least until some Commission on Nomenclature decrees.

In the latest sequel to this saga (*Nature* **280**, 468; 1979), Bennett and Stenbuck report that ankyrin is firmly linked to band 3 in detergent extracts of spectrin-depleted red cell membranes. (It should be noted that some band 4.2 is also associated with band 3 in these extracts. The data presented favour the author's interpretation of a direct linkage between ankyrin and band 3, but do not completely exclude the possibility that band 4.2 is interposed between these two proteins). The band 3 molecules involved (10–20% of the total) are apparently identical to the principal band 3 protein which functions as an anion exchange channel. Unlike ankyrin this protein spans the lipid bilayer and is presumably the true site of skeletal anchorage. Ankyrin is thus not an anchor but an anchor chain — a protein that links spectrin and band 3. In fact it may function generally to connect cytoskeletal elements and integral membrane proteins since, unlike spectrin (see below), it is apparently present in many types of cells (Bennett & Stenbuck *op. cit.*).

The electron microscopic techniques used to visualise the band 4.1 binding site have also been applied to ankyrin (Tyler & Branton *op. cit.*) and are even more successful since ankyrin is a larger molecule (a $80 \text{ \AA} \times 95 \text{ \AA}$ pyramid) than band 4.1 (a $60 \text{ \AA}$ sphere). These studies clearly show that ankyrin binds to a site $200 \text{ \AA}$ distal to the end of spectrin involved in the dimer-tetramer interaction. The stoichiometry of this linkage (whether there are one or two ankyrins per tetramer) remains to be determined.

Membrane skeletal model

Taken together, this information provides a fairly clear picture of how the principal skeletal proteins are probably organised (Fig. 1). Certain general points remain to be established (for example, the stoichiometry of the spectrin-actin-band 4.1 and spectrin-ankyrin-band 3 complexes and the physical state of actin), and many structural details must still be pursued, but the general framework appears to be taking shape. It must be emphasised however,

Halley's Comet in ancient history

from David W. Hughes

RECENT books on comets state categorically that the first definitely recorded return of Halley's comet was that of 240 BC, some adding as a postscript that there is a vague possibility that comets described in 613 BC and 467 BC may also have been P/Halley (the P/prefix indicating a periodic comet). It now seems, however, that observations go back considerably further. Y. C. Chang of the Purple Mountain Observatory, Nanking, China finds that the earliest astronomical record of the comet in the historical annals of his country described the apparition of 1057 BC. Reasons for this assertion, together with other early Chinese observations, are published in Chang's recent paper in *Chinese Astronomy* (3, 120; 1979) (a translation of *Acta Astr. Sinica* **19**, 109; 1978).

The problem of recognising the historical record as a description of Halley's comet has been approached in two ways. The first relies on celestial mechanics to produce an accurate analysis of the orbital evolution of the comet. Chang started with the position and velocity vectors of Halley's comet and the eight major Solar System planets for a specified epoch (Julian day 2430000.5). The cometary results were taken from Brady and Carpenter (*Astr. J.* **76**, 728; 1971) who based their findings on 3085 observations of P/Halley made by 60 observatories over an unbroken period of 20 months during the 1910 return.

The equation of motion of the comet around the Sun was then integrated, taking into account gravitational perturbations by the planets. This enabled the perihelion passage times and orbital elements of P/Halley to be established for the previous 3000 years. So Chang knew when and where in the sky the comet would have appeared. The planetary perturbations produce changes in the orbital period of the comet, which was for example about 72.9 years between 762 BC and 689 BC, and as long as 79.7 years between AD 1222 and AD 1301. The inclination of the orbit has decreased by about 1.5° over 3000 years, and during this time the argument of perihelion has changed from 77° to 112° and the longitude of the descending node from 18° to 58° . A more important parameter is the maximum brightness of the comet.

This seems to change by about 2.7 magnitudes per millennium, indicating that P/Halley will reach a maximum magnitude about +0.5 at its next apparition in 1986, whereas it was about -7.7 in 1057 BC — brighter than any of the planets.

Brady (*Pub. Astr. Soc. Pacific* **83**, 314; 1972) also studied the orbital evolution of P/Halley and suggests that the present planets could not account for all the observed changes. To overcome this difficulty he predicted the existence of another planet at a distance of 64 astronomical units (AU) from the Sun, with a mass three times that of Saturn. Chang does not confirm this hypothesis and finds that the known planets account for the observed perturbations. Some astronomers have also proposed that there is a cloud of comets about 50 AU from the Sun having a total mass similar to that of the Earth. Even though P/Halley, with its aphelion distance of 35 AU, would spend a considerable time near this cloud, Chang again finds that such a cloud is unnecessary when it comes to accounting for the perturbation of P/Halley.

Chang's second approach was to peruse the Chinese astronomical records, the most complete historical records in existence, for accounts of Halley's comet. The new account came to light in a chapter on military strategy in the *Book of Prince Huai Nan*. This recounts how a comet was seen in the east with its tail pointing to the northwest at the time when King Wu marched on Zhou of Yin. The book also gives the position of Jupiter at that time. There are three schools of historical thought about the time of Wu's campaign, favouring 1122 BC, 1075–1055 BC or 1027 BC. All agree however, that it occurred in the first two months after the winter solstice. By combining these factors Chang concluded that the campaign and the observation of Halley's comet took place in the winter of 1058/57 BC, so our next observations of P/Halley in 1985/86 will not be the thirtieth, as previously thought, but the forty-first recorded apparition.

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that the true skeleton is undoubtedly more complicated than the model shown in Fig 1. Skeletal proteins like bands 4.2, 4.9 and 7 are not included in the model since too little is known about their location and function. Band 4.1 must attach to the bilayer in some way since most of it remains on the membrane when spectrin and actin are extracted at low ionic strength. And

spectrin chains must often cross each other since the average spacing between ankyrin-linked band 3 dimers ($\sim 375\text{--}530 \text{ \AA}$) is considerably less than the average length of a spectrin tetramer ($\sim 2000 \text{ \AA}$).

Phosphorylation

Most of the membrane skeletal proteins

are phosphorylated in the intact red cell (Plut *et al. Eur. J. Biochem.* **82**, 333; 1978). Both cyclic AMP-dependent (ankyrin, bands 4.1, 4.9 and 7) and cyclic AMP-independent (spectrin, band 3) kinases are involved. In spectrin, the best studied example, four sites are labelled in a cluster near one end of the band 2 subunit (Harris *et al. Fed Proc.* **37**, 1507; 1978; Anderson *op. cit.*). This is probably the end of the molecule where actin and band 4.1 bind since interactions between actin and crude spectrin preparations are phosphorylation dependence in more purified systems (Brenner & Korn *op. cit.*; Cohen & Branton *op. cit.*). Nevertheless, Pinder's results strongly suggest that phosphorylation does modulate spectrin-actin-4.1 interactions in some way. Presumably it is also functionally important to the other phosphorylated skeletal proteins. Deciphering these functions and how phosphorylation affects them is probably the major unsolved issue in the field.

Evolution

Spectrin (and by implication a spectrin network) is present in all mammalian and avian red cells but has not been detected in

other cell types. Pinder and her associates have even found a spectrin-like molecule, immunochemically homologous to human spectrin, in red cells from primitive invertebrates (*FEBS Lett.* **92**, 278; 1978). This implies that spectrin has evolutionarily critical functions and, since its only known functions relate to the membranes vesiculate frenetically when evolutionarily critical structure. This is understandable from the chauvinistic perspective of the spectrinologist; red cell membranes vesiculate frenetically when spectrin is genetically absent (Greenquist *et al. Blood* **51**, 1149; 1978) or purposely removed and presumably have required support from their inception. What is not clear is how other cells survive without it! Perhaps analogous proteins have evolved to stabilise their membranes but, if so, they remain to be identified. Alternatively nonerythroid cells may have found their ubiquitous cytoskeletal scaffolding sufficient for membrane support while red cells, anxious to discard their intracellular organelles to accommodate haemoglobin, were forced to invent spectrin. Whatever the reason, it remains a mystery (at least to me). □

genotypically normal muscle in a *dy/dy* C57BL × +/+ 129 mosaic develop the typical dystrophy, non-myogenic origins of the disorder will be proved. This genetic innovation may resolve a long-standing controversy in neurobiology.

Animal mutants continue to provide valuable insights for developmental neurobiology. A dysmyelinating mutant, for example, the *shiverer* mouse, lacks immunochemically detectable myelin basic protein, according to N. Baumann (Salpêtrière, Paris) and A. Tsukada (Keio University, Tokyo). The compaction of lamellae is abnormal, but myelin is assembled despite the absence of basic protein, and the animal survives. A whole series of dysmyelinating mutants affecting central and/or peripheral myelination is now under comparative neurochemical investigation.

Invertebrates, especially nematodes (*C. elegans*) and *Drosophila*, are well-suited for genetic studies of certain limited kinds of behaviour. However, despite extensive anatomical and genetic analyses, neurochemical findings are negligible in nematodes and are only just emerging in *Drosophila*. Y. Dudai (Weizmann Institute, Rehovoth, Israel) described current studies of the *Drosophila* learning mutant 'dunce', which appears to lack one isoenzyme of cyclic AMP phosphodiesterase. The gene for this enzyme maps on the X chromosome to exactly the same position as the *dunce* mutation. Knowledge of the chromosome map and availability of deletion mutants made possible this correlation. Direct assays (D. Byars, Cal-Tech) indicate raised cyclic AMP concentrations in these flies. Obviously, there is still a gap between finding a phosphodiesterase deficiency and explaining the defect in learning olfactory differences.

Human mutations have long fertilised neurochemistry, with Tay-Sachs disease as the classic example. Gangliosides were discovered in brain tissue from patients with Tay-Sachs disease (TSD), who accumulate large amounts. The sequential nature of normal degradation of sphingolipids was revealed by recognition and analysis of a whole series of human lipid storage diseases, each due to a single gene mutation and resultant enzyme deficiency.

K. Sandhoff (University of Bonn) demonstrated anew that studies of these mutations can advance basic biochemistry of the brain. He described an endogenous activator protein essential to the physiological action of hexosaminidase A (deficient in TSD) on lipid soluble substrates in micelles *in vitro*. Presumably, this activator is crucial for Hex A action on lipids within membranes *in vivo*. In clinical neurochemistry, the activator protein

Genetic approaches to neurobiology

from Gilbert S. Omenn & John P. Blass

THE concepts and techniques of genetics are being wedded to those of the neurosciences with increasing success. Studies of genetically defined lesions in invertebrates, mice, humans, and cultured cells are providing descriptive and pathogenetic information about complex brain functions and behaviours. Several productive new lines of research at this interface were reported at an International Society for Neurochemistry (ISN) meeting in Greece*.

Deliberate construction of mosaic or mutant mice now offers a powerful way to investigate models of human disorders and to distinguish whether dysfunction is intrinsic or extrinsic to the nervous system or muscle. B. Mintz (Institute for Cancer Research, Philadelphia), who pioneered the use of allophenic mice (formed by aggregating cells from two 8-cell embryos from two different pregnant mice, hence tetraparental), has constructed such mice using mouse teratocarcinoma (TC) cells in place of one pair of parents. The TC cells serve as a remarkable vehicle for introduction of specific marker or mutant genes, obtained by any of the techniques of somatic cell genetics including mutagen treatment and selection, recombinant DNA techniques, and cell fusion. Mintz

has created an HGPRT⁻/HGPRT⁺ mosaic mouse from a normal embryo plus HGPRT-TC cells. Completely HGPRT⁻ progeny generated from these mice will be the first true animal model for the fascinating and perplexing human inborn error of metabolism, Lesch-Nyhan disease. In Lesch-Nyhan disease, deficiency of HGPRT (hypoxanthine guanine phosphoribosyltransferase) as a result of a single structural gene mutation leads to complex behavioural pathology, with compulsive aggressiveness, and neurological abnormalities. HGPRT alleles with varying residual enzyme activity could be used to generate a graded series of HGPRT deficiencies for neurochemical and behavioural correlations and it is likely that a whole array of specific mutant mice will be constructed in the years ahead.

A. Peterson (McGill University, Montreal) described allophenic mosaic mice (strains C57BL and 129) constructed to test whether muscular dystrophy (*dy/dy*) in the mouse is of primary muscle or primary neurogenic origin. Mosaics between strains 129 and C57BL were observed to have muscle entirely of 129 origin (according to marker enzymes), while the rest of the animal is mosaic. Thus, Peterson could construct mosaic mice with *dy/dy* muscle; such genotypically abnormal muscle proved to be entirely normal on examination, indicating an extramuscular mechanism. If the complementary mice with

*Symposium on Genetic Approaches to Neurobiology and Neurological Disorders, University of Patras School of Medicine, Patras, Greece, 30-31 August, 1979; official satellite Symposium of Congress of International Society for Neurochemistry, Jerusalem, 2-6 September, 1979. Organisers were S. Doxiadis, P. Kafatos, E. Kouvellas, A. Simopoulos, J. P. Blass and G. S. Omenn.

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effect can explain the previously perplexing patterns of accumulation of gangliosides and globosides in patients with the various forms of TSD; Hex A, but not Hex B, is strikingly activated. Finally, three TSD patients with apparently normal Hex A activity (AB variant) were found to lack the Hex A-activator protein. It is very likely that analogous activator proteins specific for sulphatides and other lipids exist. They may prove to have interesting protein structures, and a sulphatide-specific activator protein may account for known cases of metachromatic leukodystrophy (MLD) with apparently normal activity of the arylsulphatases.

These highlights illustrate the power and sensitivity of genetic approaches to studies of complex functions of the nervous system and behaviour.

As this Symposium was an integral part of the Jerusalem meeting of the ISN, it is significant to note that Professor Ekram Abdel Salam of Cairo University and Professor Yadin Dudai of the Weizmann Institute were both active participants. In the near future, we expect that such contacts will be made directly in Egypt and in Israel. □

The end of the Cretaceous

from A. Hallam

It is widely recognised that the end of the Cretaceous witnessed one of the most significant mass extinction events in Earth's history, both on land and at sea. Whereas certain groups like the ammonites had been in decline for some time, others such as the dinosaurs and planktonic foraminifers were still flourishing until the end of the period. The many hypotheses that have been proposed to account for this striking extinction event fall into two categories, respectively invoking extra-terrestrial and terrestrial causes.

The extra-terrestrial or 'astronomical' hypotheses involve increased levels of some form of unpleasant radiation from outer space, perhaps at times of geomagnetic reversals. They have been strongly criticised and are not widely accepted today but the general notion cannot yet be decisively discounted and has been recently revived by Reid *et al.* (*Nature* 259, 177; 1976; 275, 489; 1978), who suggest increased ultraviolet radiation at the Earth's surface as a result of breakdown of the protective ozone belt, caused by increased solar proton flux or supernova explosions. One of the strongest arguments against such radiation events to account for various mass extinctions in the past is that

Interstellar molecules

from B. Zuckerman

AFTER a decade of explosive growth, the study of interstellar molecules has reached a certain level of maturity. That seemed to be the picture that emerged from a recent symposium at which the organisers rather successfully blended a combination of astronomers, physicists, chemists and spectroscopists. That they were perhaps not entirely successful was suggested during the presentation of one European astronomer, when a "thank you" from the speaker directed to the slide projectionist woke up a sizable fraction of the audience who then proceeded to deliver an unexpected round of applause.

The startling new results that characterised the early 1970s were absent from this symposium. Still there are many controversies and mysteries yet to be unravelled. In spite of the very low temperatures and fairly quiescent states that characterise most interstellar molecular clouds there is evidence of surprisingly energetic phenomena hidden deep inside some clouds. S. Beckwith (Cornell University), T. Geballe (California Institute of Technology), N. Scoville (University of Massachusetts, Amherst) and D. Hollenbach (NASA Ames Research Center) discussed high velocity gas and shock waves in the cloud behind the Orion Nebula. The very large energies associated with the high velocity gas imply the existence of massive stellar winds or an explosive event (perhaps a supernova) inside this molecular cloud.

Even for the more typical molecular cloud, J. Silk (University of California, Berkeley) and C. Norman (University of Leiden) suggested that support against gravitational collapse can best be provided by energetic winds from new stars similar to the Sun that have just formed inside the clouds. That molecular clouds are

somehow stabilised against free-fall gravitational collapse now seems generally, although perhaps not universally, accepted. Indeed the present controversy rages around the question of whether the giant molecular cloud complexes 'live' for at least a few times 10^8 years (P. Solomon, State University of New York, Stony Brook) or 'only' a few times 10^7 years (L. Blitz, University of California, Berkeley). In the former case the molecular clouds are well distributed throughout the inner disk of our Milky Way Galaxy, in the latter case they are confined to the spiral arms. Future observations of carbon monoxide in external galaxies should resolve the matter.

A renaissance in this field awaits development of sensitive new receivers especially for wavelengths ≤ 1 mm to be used with large new mm wavelength telescopes and interferometers presently under construction or envisaged. Previews of some of the exciting results to be expected at high frequencies were given by P. Vanden Bout (University of Texas) and by T. Phillips (Bell Telephone Laboratories). Phillips reported submillimetre observations of CO (at 461 GHz) and of H₂O (at 380 GHz) obtained from NASA's Kuiper Airborne Observatory.

One of the fundamental limits in this field is the total number of different interstellar molecules that may eventually be found by radioastronomers. P. Thaddeus (Goddard Institute for Space Studies, New York) pointed out that 52 interstellar molecules have now been identified but, from considerations of the limits set by confusion due to overlapping weak lines from various molecules, he estimated that probably only about three times this number would ever be identified. Even so, the microwave spectroscopists will have their work cut out for them if we are to assign the observed transitions properly. □

*The Symposium on Interstellar Molecules was held at Mont Tremblant, Quebec on 6-10 August and sponsored by the International Astronomical Union in conjunction with the Herzberg Institute of Astrophysics and the National Research Council of Canada.

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terrestrial floras were comparatively unaffected. It is noteworthy therefore that Krassilov (*Palaeontology* 21, 893; 1978) has recorded an abrupt change in terrestrial floras across the Cretaceous-Tertiary boundary.

The 'terrestrial' group of hypotheses usually postulate some form of sea level and/or climatic change. There is good stratigraphic evidence of a major eustatic regression at the end of the Cretaceous, and such regressions plausibly account for a number of mass extinction episodes of shelf faunas in the Phanerozoic, but it is not so evident why planktonic and terrestrial groups should be seriously

affected. One ingenious solution to this problem was proposed some years ago by Tappan (*Palaeogeog.*, *Palaeoclimatol.*, *Palaeoecol.* 4, 187; 1968). She postulated that the production of marine phytoplankton controlled the oxygen and carbon dioxide levels of the atmosphere through time, and that changes in relative and absolute abundance of these organisms had extensive effects on contemporary biota. Following the major late Cretaceous transgression which preceded the end Cretaceous regression, the influx of nutrient supply from land dropped as continents were reduced to base level. This and other associated processes led to a marked reduction in marine phytoplankton and a consequential

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increase of atmospheric carbon dioxide and decrease of oxygen (due to reduced photosynthesis). These atmospheric changes were too much for the dinosaurs to cope with, though estivators and hibernators such as the crocodiles and turtles were able to survive into the Tertiary.

Data from new fields of research have now been brought to bear on the problem. Geomagnetic reversals as recorded in sedimentary sequences both on land and under the ocean can be used to add precision to stratigraphic correlation based on fossils. A biostratigraphically complete sequence of Upper Cretaceous and Palaeogene pelagic limestones at Gubbio, Italy has produced a record of geomagnetic reversals that closely matches the marine magnetic anomaly sequence (Alvarez *et al.*, *Bull. geol. Soc. Am.* **88**, 367; 1977). A detailed analysis of sedimentation rates at Gubbio suggests that the Maastrichtian — Danian faunal overturn might have happened very rapidly, of the order of 10,000 years or less (Kent *Geology* **7**, 66; 1979). Palaeontological work on a comparably complete sequence of pelagic deposits at Zumaya, Spain suggests that environmental stresses were first felt by planktonic foraminifera about 10,000 years before the end of the Maastrichtian, following which occurred the major crisis involving extinction of globotruncanid foraminifera and a drastic change in the nannoplankton flora (Percival & Fischer *Evol. Theory* **2**, 1; 1977).

Interest naturally focuses on whether or not the dinosaur extinction was coincident in time with that of the planktonic groups. Only if it was precisely coincident can the case for a drastic extra-terrestrial event seriously be upheld. Butler *et al.* (*Nature* **267**, 318, 1977) undertook a magnetostratigraphic study of a sequence of terrestrial sediments in New Mexico which they argued exhibited an unbroken transition across the Cretaceous — Tertiary boundary. By correlating with the Gubbio sequence they inferred that the dinosaurs became extinct slightly later than the planktonic foraminifera, perhaps by as much as half a million years. This correlation has been criticised by Alvarez *et al.* (*Geology* **7**, 66; 1979) who suggest that an unconformity might be present in the New Mexico sequence. If so, the marine and terrestrial extinction events could indeed be synchronous. Further information on the issue has recently been published by Lerbekmo *et al.* (*Nature* **279**, 26; 1979) in another magnetostratigraphic study of a terrestrial sequence in Alberta. Their data indicate an approximate synchronicity of dinosaur and foraminifera extinctions just below anomaly 29, the maximum error being about 100,000 years.

Oxygen isotope data from foraminifera in deep sea cores has been used to monitor water temperature changes across the

Cretaceous — Tertiary boundary. McLean (*Science* **201**, 401; 1978) cited isotope work on benthic forams indicating a late Maastrichtian warming event and went on to propose a novel interpretation of dinosaur extinctions by a temperature rise rather than a temperature fall, as had been frequently speculated previously. He failed, however, to cite other isotope work indicating just the reverse, a slight cooling through Maastrichtian time (Savin & Douglas *Bull. geol. Soc. Am.* **84**, 2327; 1973; Saito & Van Donk *Micropalaeontology* **20**, 152; 1974). This latter interpretation is indeed more in accord with what is known from the terrestrial plant record (for example Krassilov *Palaeogeog., Palaeoclimatol., Palaeoecol.* **17**, 157; 1975).

The most recent isotope data is reported from an exceptionally well documented Atlantic core by Thierstein and Berger (*Nature* **276**, 461; 1978). The oxygen isotope values remain more or less stable through the Maastrichtian part of the core

and then exhibit a sharp change into the basal Danian. This can be interpreted as signifying a sudden water temperature increase (in which case it comes too late to support McLean) or as a salinity decrease. Thierstein and Berger favour the latter interpretation and cite the evidence as support for their hypothesis that the extinction event was caused by a large-scale invasion of the ocean by low salinity water from a previously isolated high latitude basin. Their completely original idea on mass extinctions is likely to prove as controversial as many others on this subject, especially as the existence of extensive high latitude ice masses at the end of the Cretaceous seems unlikely for a number of reasons, and it is hard to see how otherwise the requisite large volume of low salinity water could be created. Despite all the new data and ideas it seems probable that the extinctions at the end of the Cretaceous will continue to intrigue us for some time to come.

□

SEASAT's short-lived mission

from T. D. Allan

ON 10 October, 1978, after little more than one hundred days of operation, the transmissions from SEASAT-1 — the first satellite dedicated to a study of the ocean surface — were brought to an untimely end by a catastrophic short circuit in the power system.

The declared first objective of SEASAT's mission was to test whether satellite microwave sensors could provide data of a high enough accuracy to be incorporated into existing systems for forecasting and charting global ocean conditions. It was an ambitious project which had been planned for over 10 years by oceanographers and NASA engineers. The accuracy criteria laid down as design goals for the satellite sensors fell little short of the accuracies that can presently be achieved by ships and surface buoys. If SEASAT's sensors can be shown to have achieved these precisions under different ocean conditions then routine monitoring of the sea surface from satellites becomes a real and exciting alternative to the sparse data of variable quality currently obtainable from surface vessels.

The major difference in SEASAT's payload from previous environmental satellites was the concentration of radar sensors with the all-weather capability that is essential for repetitive surveys of sea areas mostly covered by cloud. Of the four

microwave sensors carried on SEASAT — three active and one passive — the radar altimeter had some space heritage, previous versions having been flown on Skylab and GEOS-3. However, no instrument had even measured its own orbiting height to an accuracy of ± 10 cm which was the precision required to provide useful information on ocean currents, tides and surface waves.

The radar scatterometer was designed to satisfy the weather forecasters' requirements for a comparatively dense grid of wind field observations. The instrument swept out swathes of over 500 km on either side of the spacecraft's sub-orbital track and related ocean backscatter to surface winds. Further information on wind speed was provided by the passive microwave radiometer which measured brightness temperature at five frequencies in the range 6.6 — 37 GHz.

The most controversial of SEASAT's suite of sensors was the synthetic aperture radar (SAR) which, operating at 1.3 GHz (23 cm wavelength), was designed to produce an image of the sea surface with a spatial resolution of 25 m. The data rate required to achieve such a fine resolution is so high (approximately 100 megabits s⁻¹) that on-board recording was out of the question and SAR could only be operated in real time over areas within range of a ground receiving station. The European SAR station was at Oakhanger in Hampshire, operated by the Royal Aircraft

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Establishment (Farnborough) under an agreement between ESA and NASA.

The performance of a satellite's sensors must be assessed against reliable surface observations and the question now being asked of the SEASAT mission is whether sufficiently diverse surface data were obtained last summer to satisfy the main 'proof-of-concept' objective. A special data-gathering cruise was organised by the US National Oceanographic and Atmospheric Administration (NOAA) in the Gulf of Alaska during a 4-week period in August-September 1978. Observations of wind, waves, temperature and other parameters were made by two ships, four aircraft and an array of surface buoys. A detailed comparison between these observations and the signals from SEASAT's sensors was made recently during two workshops held at the Jet Propulsion Laboratory, Pasadena. More than 60 scientists and engineers from several oceanographic and space research centres were involved and the preliminary results for each sensor have been reported in *Science* (204, 1405-1424; 1979).

The scatterometer task group had evolved three candidate algorithms which made slightly different assumptions in relating the measured scattering coefficient to the wind vector as a function of incidence angle, azimuth angle and polarisation. Since these were largely constructed from a limited set of aircraft measurements it is not surprising that the satellite models required some modifications especially with regard to determining wind direction unambiguously. An analysis of some 30 comparisons showed the scatterometer winds to be biased high by 1.9 m s^{-1} with a standard deviation about this bias of 1.8 m s^{-1} . When the scatterometer values were compared with wind fields produced by kinematic analysis techniques (as opposed to spot measurements) wind speed differences were in the range 1.1 – 5.2 m s^{-1} (scatterometer higher) and for direction the means were less than 10° with standard deviations of approximately 20° . Wind speeds were generally low throughout the experiment and part of the observed differences may be attributed to errors in the surface spot observations.

The scanning microwave multi-channel radiometer (SMMR) measured Earth's radiation at five microwave frequencies at both vertical and horizontal polarisation. The algorithms to extract sea surface temperature and wind speed are correspondingly complex since they involve ten values of brightness temperature with different weightings according to frequency, to the antenna pattern, and to the amount of cloud and rain present. Over open ocean the best wind speed determinations gave a standard deviation of 3 m s^{-1} about a bias of 1.5 m s^{-1} . Sea surface temperature estimates were disappointing with a standard deviation of 1.5°C about a bias of 3 – 5°C . However,

further analysis may determine the cause of the bias and suitable correction can be made. The SST value is averaged over a footprint approximately 120 km on the side.

In validating the performance of the radar altimeter the GOASEX data were used to calibrate only significant wave height and radar backscatter; the calibration of the altimeter's height determination was made over a range in Bermuda. During its last few weeks of operation SEASAT was manoeuvred into a frozen orbit pattern which allowed it to pass over Bermuda every 3 days. The calibration range included high precision tide gauges and a laser tracking system. For four overhead passes where laser tracking was possible the weighted mean bias in the altimeter-derived height was $-50 \pm 11 \text{ cm}$ (over a height of 800 km). During the engineering assessment of the altimeter the noise in the height measurements for a 4-m significant wave height was 5–8 cm. If relative changes of this order can be detected in sea surface topography than valuable information can be obtained on ocean circulation patterns, eddies, tides, and the marine geoid. For this reason, the altimeter represents perhaps the greatest loss to the oceanographic community.

There was some lively discussion before the synthetic aperture radar was flown on SEASAT as to its ability to image surface waves. SAR sweeps out a 100 km swathe centred about a point 250 km to the right of the satellite's sub-orbital point. Each target remains in the radar beam for about 2 seconds and it was argued that in this time the movement of the shorter capillary waves which scatter the L-band energy could be more than enough to blur the return image. And since the detection of the larger gravity waves was seen to depend on their modulation of the shorter waves, it seemed doubtful if they could be detected. In the event, SAR produced several images of swell waves not only in the Gulf of Alaska but in other ocean areas including those covered by the Oakhanger station. Wave directions can be estimated to a few degrees and wave length greater than about 100 m appear to show clearly, given a certain minimum significant wave height (apparently around 2 m), certain minimum wind conditions and, up to the present, a wave direction predominantly across the satellite track. Apart from imagery of surface swell the SAR record analysed to date has shown remarkable examples of internal wave trains, surface pollutants, ice features in the Arctic, and details of shallow sand banks in the Channel and other shallow water areas around the UK. Its fine resolution (25 m against Landsat's 80 m) and its independence from adverse weather conditions have now aroused the interest of land surveyors and geologists.

Before the launch of the spacecraft the SEASAT Users Research Group of Europe (SURGE) had submitted a list of proposed experiments to NASA and as a result SAR

was activated over a number of European sites. The most important of these was JASIN — a Joint Air-Sea Interaction experiment in a comparatively small area close to Rockall in which up to 14 research vessels, 3 aircraft and 35 buoys took measurements in the period mid-July to mid-September. Although SEASAT's early demise is proving increasingly regrettable as its results become available, it was a piece of extremely good fortune that one of the most intensive research programmes of sea-surface observations took place during its short life. Now that the sensor algorithms have been refined and modified following GOASEX, the JASIN data should allow a comprehensive assessment of the performance of each microwave sensor. It may still be possible to go a long way towards reaching SEASAT's first objective — to prove the concept of reliable remote sensing of the seas from satellites. □



100 years ago

It may interest our readers to know the elevations which at present are reached by lines of railway in different parts of the world. The Apennine Railway reaches its highest point at an elevation of 617 metres above sea-level; the Black Forest Railway ascends to 850 metres, the Semmering line to 890, the Caucasus line to 975 metres. The St. Gothard tunnel is 1,154 metres above sea-level; the railway across the Brenner reaches 1,367 metres; the Mont Cenis Railway ascends to 1,338 metres, the North-Pacific line to 1,652, the Central-Pacific to 2,140, and the Union-Pacific to 2,513 metres. The highest of all is the line across the Andes, which reaches an elevation of 4,769 metres.

We have on our table the following works: — "The Spiders of Dorset," Rev. O. Pickard Cambridge; "Chemical and Geological Essays," by T. Sterry Hunt (Trübner); "Deaths in Childhood," Dr. Aeneas Munro (Smith, Elder, and Co.); "The Silk Goods of America," W.C. Wyckoff; "Structural Botany," Dr. Asa Gray (Trübner); "Luxurious Bathing," A. W. Tuer (Field and Tuer); "Phreology Vindicated," A. L. Vago (Simpkins); "On the Diffusion of Liquids," J. H. Long (H. Laupp); "Reform Essays on Incentive Religion and Warfare," "Farming for Pleasure and Profit" (Poultry Keeping), Arthur Roland (Chapman and Hall); "Manual of Practical Anatomy," J. Cossar-Ewart (Smith, Elder, and Co.); "Rays from the Realms of Nature," Rev. James Neil (Cassell); "Jack's Education; or, How He Learnt Farming," Prof. Henry Tanner (Chapman and Hall); "Vocal Physiology and Hygiene," Gordon Holmes (Churchill); "Fauna der Gaskohle und der Kalksteine der Perm Formation Böhmens," Dr. Ant. Fritsch.

From *Nature* 20, 9 October, 563; 1879.

review article

Incommensurable structures

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The discovery of incommensurably distorted structures has important implications for the study of structural phase transitions.

ON 8 June, 1912 von Laue and coworkers presented a paper entitled *Interferenz-Erscheinungen bei Roentgenstrahlen* in which a theory of the diffraction of X rays by a periodic lattice of atoms was outlined¹. In 1913, Bragg² reported the structures of four alkali halides determined by X-ray techniques. Since these early experiments many thousands of crystal structures have been determined and the concepts of crystal periodicity and symmetry have had a central role in the development of solid state physics. According to the conventional view crystalline solids are composed of plane-sided cells which are stacked on a regular lattice of sites. Depending on the symmetry of the lattice and of the arrangement of the atoms within each cell, a crystal is assigned to one of the 230 possible space-symmetry groups. By definition, when any of the symmetry elements which comprise the space group are applied to the crystal it is mapped into itself and its physical properties are unchanged. Apart from some pathological and (as we shall see) interesting exceptions the symmetry groups encountered in practice comprise rotations, reflections and translations in our three-dimensional world. Nevertheless, it is simpler, and more relevant to this article, to consider a one-dimensional crystal (chain) such as that depicted in Fig. 1A. Evidently, left-right translation of the chain by any multiple of the unit-cell side, a , leaves the chain unaltered, as does inversion (reflection) through a suitably chosen point.

Structural transformations

Many solid materials have been found to change their crystal structure when some externally applied perturbation such as temperature is varied. Structural transitions of this type, particularly those which occur continuously, have been the subject of intensive study during the past 20 years or so³. One type of continuous transition of the so-called displacive type, can be visualised with the aid of Fig. 1. Suppose that the atoms of the chain are subjected to longitudinal (left-right) displacements whose amplitudes vary sinusoidally in space as shown in Fig. 1B. The atomic positions which result from this modulation will be as depicted in Fig. 1C. A transition between the distorted chain and the high-symmetry uniform chain occurs when the modulation amplitude vanishes. This amplitude is thus the order parameter of the transition and plays an analogous role, for example, to the magnetisation in a transition between ferromagnetic and paramagnetic states. The fact that the modulation vanishes continuously at the transition temperature, T_c , gives rise to the designation 'continuous transition'.

Much of the detailed information which has been obtained about real structural transformations has been derived from diffraction experiments with X rays or neutrons⁴. Once again, these experiments may be understood with the aid of a one-

dimensional example. Suppose that a beam of X rays of wave vector $\mathbf{k}_0$ (wavelength $\lambda_0 = 2\pi/k_0$) impinges on the one-dimensional chain depicted in Fig. 2A. Each atom of the chain acts as a scattering centre and radiates X rays of wave vector $\mathbf{k}$ where $|\mathbf{k}| = |\mathbf{k}_0|$. The interference between the various scattered beams will be constructive only if the component of $\mathbf{k}_0 - \mathbf{k}$ in the y-direction is a multiple of $2\pi/a$. Generally, the quantity $\mathbf{k}_0 - \mathbf{k}$ is known as the scattering vector and is denoted by $\mathbf{Q}$. In the reciprocal space defined by the cartesian components of $\mathbf{Q}$, diffraction from the one-dimensional chain occurs only for $Q_y = 2\pi n/a$ where n is an integer. The values of $\mathbf{Q}$ -components (Q_x, Q_z) perpendicular to the chain are immaterial. Thus the loci of allowed scattering vectors comprise a series of sheets in reciprocal space, as shown in Fig. 2B. These arguments may be easily extended to both two- and three-dimensional scattering arrays. A two-dimensional array (Fig. 2C) may be regarded as a chain (in the x-direction) of chains (in the y-direction). Its diffraction pattern is therefore characterised by the intersections of two mutually perpendicular sets of planes in reciprocal space. As shown in Fig. 2D, this gives rise to rods of scattered intensity. Extending the argument to one more dimension one finds that the diffraction pattern of a three-dimensional crystal is a three-dimensional array of points in reciprocal space. While such an array of Bragg points is the most commonly observed form of diffraction, patterns similar to those depicted in Fig. 2 are not simply figments of the imagination, but can (as will be shown) be observed in the laboratory.

As we have seen, the diffraction pattern of the uniform chain in Fig. 1A consists of a series of sheets in reciprocal space separated by multiples of $2\pi/a$. The diffraction pattern of the modulated chain (Fig. 1C) may be deduced in an analogous manner. As a result of the choice of the modulation wavelength as $4a$, every fourth atom is displaced by the same amount. The distorted structure can thus be described in terms of a unit cell which is four times larger and contains four times as many atoms as the original one. Accordingly, the diffraction pattern of the distorted phase consists of sheets separated by $\pi/2a$ rather than by $2\pi/a$. That is, three equally spaced sheets are inserted between each pair shown in Fig. 2B. Evidently, the intermediate sheets, known as satellite reflections, disappear when the amplitude of the lattice modulation tends to zero. (When all sheets are equally spaced, the intermediate ones are generally known as superlattice reflections. The term 'satellite reflection' is used here to avoid a proliferation of terminology.) Thus, the intensities of the satellite reflections serve as a measure of the modulation amplitude, that is, of the order parameter of the transition.

The previous example is a prototype for most of the continuous structural transitions which have been studied by diffraction

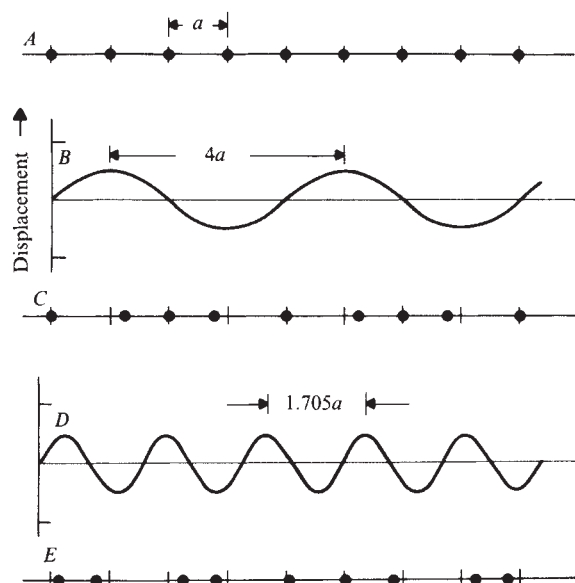


Fig. 1 A, a uniform chain of atoms; B, a commensurable modulation; C, distorted chain which results when the modulation of B is imposed on the chain in A; D, an incommensurable modulation; E, distorted chain which results when the modulation of D is imposed on the chain in A. In rows A, C and E the tick marks represent the positions of the undisplaced atoms.

techniques during the past 15 years or so. In general, the modulations which have been observed apply to a three-dimensional crystal and the diffraction patterns consist of a lattice of points rather than sheets in reciprocal space. Nevertheless, the distinction between main Bragg points and satellite reflections remains the same. The canonical example⁵ of this type of phase transition is that which occurs at 105 K in SrTiO_3 . Above the transition temperature the crystal structure, shown in Fig. 3B, consists of Ti atoms at the corners of a cubic cell surrounding a Sr atom at the centre of the cell. Each Ti is surrounded by oxygen atoms located at the apices of the octahedra drawn in Fig. 3B. In the low-temperature (distorted) phase the oxygen octahedra, which are linked by a common oxygen atom at their corners, are rotated in the senses indicated by the arrows. This cog-wheel type of rotation is shown more clearly in Fig. 3A, where the positions of the oxygen atoms at low temperature are indicated by circles while their positions above the transition are indicated by hatched circles. Evidently, the low temperature distortion is periodic but the modulated variable is the angle of rotation of the octahedra rather than the longitudinal atomic displacement of Fig. 1. The periodicity of the modulation (see Fig. 3) is, however, enough to cause a doubling of each unit-cell side.

Most previous reports on structural transitions described systems in which the unit-cell size either remained the same or was changed by a simple multiple at the transition. However, it is certainly not *a priori* necessary for a lattice modulation to have a periodicity which is a rational multiple of a unit-cell side. An example of a modulation which does not satisfy this simple criterion and which is thus incommensurable with the undistorted lattice is shown in Fig. 1D. (The term incommensurate has been used in this context, probably without malice. Incommensurate is defined as not adequate.) The atomic arrangement in the incommensurable phase is depicted in Fig. 1E. This phase has the remarkable property that no two atoms are displaced by the same distance from their positions in the undistorted phase. It is thus impossible to find any lattice translation which maps the crystal into itself. For the case of a three-dimensional crystal this means that the crystal does not belong to any of the 230 space groups mentioned earlier. In spite of this apparent lack of symmetry, the diffraction pattern of an incommensurably modulated structure is relatively simple. Just as for a commensurable distortion there are principal reflections, separated by $2\pi/a$, and satellites, whose positions depend on the wave vector q_0 of the modulation ($q_0 = 2\pi/1.705a$ for Fig. 1D, E).

The satellites are separated from the principal reflections by multiples of q_0 .

Symmetry classifications of incommensurably modulated structures have been developed⁶. The distorted chain in Fig. 1E is mapped into itself by an operation which translates the chain to the right by a distance na and changes the phase of the modulation by q_0na . Thus, the translational-symmetry group of the crystal consists of operations which translate the crystal and, at the same time, slide the distortion wave with respect to the crystal. This means that the symmetry operations act in a hyperspace whose dimensionality is greater than that of the crystal. For a three-dimensional crystal with a single modulation wave, this hyperspace is four-dimensional and comprises the three spatial dimensions and a dimension which refers to the phase of the modulation.

Incommensurable phase transitions

The first structurally incommensurable phase transition⁷ to be identified as such was found in the insulator NaNO_2 . Subsequently, such transitions have been discovered both in metallic and insulating materials. The physical effects which drive the transitions are somewhat different in the two cases. For the insulators it is often found that competing, short-range interatomic forces of different ranges and similar magnitudes give rise to an incommensurably modulated phase. To see crudely how this may arise, consider the monoatomic chain in Fig. 1 and suppose that only elastic forces between first and second neighbour atoms contribute to the energy. The latter may then be written as

$$U = \sum_{n=1}^N \left\{ A_1 \sum_{i=\pm 1} (u_n - u_{n+i})^2 + A_2 \sum_{j=\pm 2} (u_n - u_{n+j})^2 \right\} \quad (1)$$

where u_n is the longitudinal displacement of the n th atom from its lattice site and A_1 and A_2 are the first and second neighbour harmonic force constants, respectively.

Expanding the displacements as a Fourier series

$$u_n = \sum_q u_q \cos qna \quad (2)$$

and substituting in equation (1) one finds that the energy is given by

$$\begin{aligned} U &= 4 \sum_q u_q^2 [A_1(1 - \cos qa) + A_2(1 - \cos 2qa)] \\ &= 4 \sum_q u_q^2 A_q \end{aligned} \quad (3)$$

A sinusoidally modulated structure will be stable if A_q in equation (3) has a minimum value for a non-zero wave vector q_0 . Necessary conditions for this to occur are that

$$\frac{\partial A_q}{\partial q} = 0; \quad \frac{\partial^2 A_q}{\partial q^2} > 0 \quad (4)$$

at the wave vector q_0 . These conditions imply that a modulation of wave vector q_0 given by

$$\cos q_0 a = -A_1/4A_2 \quad (5)$$

will be stable provided $A_1 < 4|A_2|$ and $A_2 \leq 0$.

A well-studied system which exemplifies the above calculation is the insulator K_2SeO_4 . In this material⁸, which transforms to an incommensurable phase at 130 K, force constants (see A_1 and A_2 above) between first- and second-neighbour layers of atoms decrease strongly with decreasing temperature above 130 K. However, third-neighbour interlayer forces increase slightly in the same temperature interval. It is thought that the competition between these short-range forces is responsible for the observed transition.

For conducting materials, interesting incommensurable transitions result from the interaction between conduction electrons and the atomic lattice. These interactions give rise to effective interatomic forces which are long-range. Although the

appearance of an incommensurable phase could be described in terms of competition between such forces, this language is unusual for metals. Rather, attention is focused on the fact that in certain cases the energy of a metal is lowered if the conduction-electron density is spatially modulated^{9,10}. Thus, a so-called charge density wave (CDW), whose wave vector is an extremal Fermi surface dimension, is set up in the conduction electron gas. The ions of the lattice respond to the CDW, in an attempt to preserve local charge neutrality, and an incommensurable lattice modulation is thereby generated. Examples of this phenomenon are to be found among the transition metal dichalcogenides¹¹, the one-dimensional charge-transfer salts (such as TTF-TCNQ¹²) and certain chain compounds (for example KCP¹³). The transition to the incommensurable phase is known as a Peierls transition.

Anharmonic effects

Although instructive, the calculation leading to equation (5) is deficient in several ways. First, equation (1) is a truncated expansion of the total energy in terms of the displacements u_n . There ought to be terms of higher order in u_n . These so-called anharmonic terms limit the magnitude of the amplitude of the incommensurable distortion which, according to equation (3), is otherwise unbounded. A second defect of equation (1) is that it is a zero-temperature approximation. At finite temperatures, the addition of the obligatory entropy term may radically alter the behaviour deduced above, and may even suppress the distorted phase entirely.

In addition to restricting the amplitude of a modulation, the anharmonic terms mentioned above have an important qualitative effect on the nature of a modulated phase. In a commensurably modulated structure, such as Fig. 1C, the anharmonic terms determine the optimum phase of the distortion (Fig. 1B) with respect to the uniform lattice (Fig. 1A). For a particular choice of phase, such as that depicted in Fig. 1A, B, in which every other atom is undisplaced in the modulated phase, the energy is minimised. This situation is unique to commensurably modulated structures. In the incommensurable case, the phase of the distortion is, by definition, different at each lattice site. The energy of the system is thus unchanged if the modulation is translated with respect to the lattice and the concept of 'optimum phase' has no meaning. Nevertheless, in any small region, the incommensurable wave of Fig. 1D causes a distortion which is very similar to that of a commensurable modulation of wavelength $5a/3$. There are even regions in which the distortion has a phase which reduces the local contribution to the energy. However, these regions are compensated by others in which the distortion has the 'wrong' phase and the local energy is large. In this situation there are two ways in which the sinusoidal modulation may be modified in order to lower the energy of (render more stable) the distorted phase. First, the amplitude of the

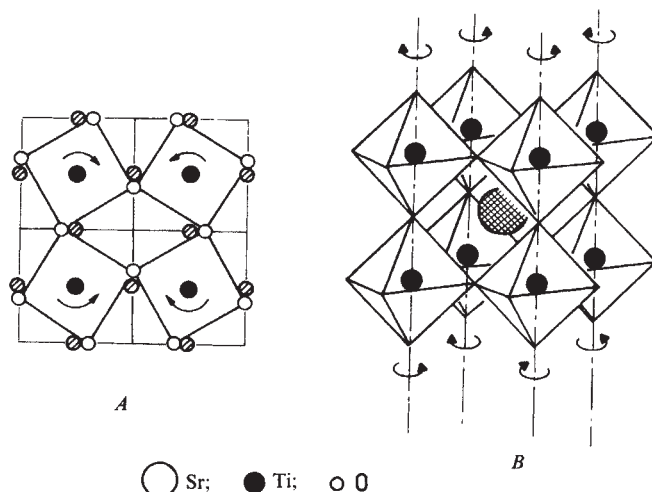


Fig. 3 A, A plan view of the structure of SrTiO₃ below the 105 K phase transition. The hatched circles represent the positions of the oxygen atoms in the high temperature (symmetric) phase. Arrows indicate the 'cog-wheel' type distortion induced by the transition. B, Three-dimensional representation of the symmetric phase of SrTiO₃.

distortion may be increased in the 'correct'-phase regions and decreased in the 'wrong'-phase regions. Alternatively, the phase may be distorted and made to vary from region to region. It is generally accepted that the latter possibility occurs most readily. To calculate the effect¹⁴ it is necessary to return to equation (2) and to replace the discrete atomic positions (na) by a continuous coordinate x . Equation (2) is then rewritten as

$$u(x) = A \cos(q_c x + \phi(x)) \quad (6)$$

where q_c is a commensurable wave vector close to the wave vector of the sinusoidal distortion deduced from equation (5). $\phi(x)$ is a phase whose variation with position, x , minimises the energy obtained when equation (6) is substituted in the anharmonic (non-truncated) version of equation (1). The equation which results from this minimisation procedure is known as the (static) Sine-Gordon equation¹⁵. This nonlinear differential equation has solutions similar to those represented by the stepped curve in Fig. 4. The straight line in this figure represents the phase variation which would correspond to the simple, sinusoidal modulation with a wave vector given by equation (5). Each step in the curve in Fig. 4 represents a sudden change of phase of the modulation. The steps can be regarded as walls separating domains in which $\phi(x)$ is independent of x and which are thus commensurably distorted. In these domains the 'optimum phase' argument applies and the anharmonic energy is thereby minimised. This reduction of energy of the system more than offsets the cost in harmonic energy which results because $\phi(x)$ does not follow the straight line in Fig. 4, but is stepped instead. Evidence for the importance of anharmonic effects and for the plausibility of the solution sketched in Fig. 4 have been obtained, for example, by Moncton *et al.*¹⁶ in their neutron scattering experiments with 2H-TaSe₂.

The phase steps in Fig. 4 are called solitons. They are an example of the solitary waves or kinks which are the solutions to many nonlinear wave equations. As an aside, one may note that solitary waves are not a new phenomenon in physics. In 1844, Russell reported a solitary water wave which he had observed in a canal¹⁷: "... rolled forward with great velocity, assuming the form of a solitary elevation, a rounded, smooth and well-defined heap of water, which continued its course along the channel apparently without change of form or diminution of speed. I followed it on horseback... and after a chase of one or two miles I lost it in the windings of the channel". The solitary wave pursued by Russell bears a singular resemblance to the derivative $d\phi/dx$ of the function plotted in Fig. 4 although, in fact,

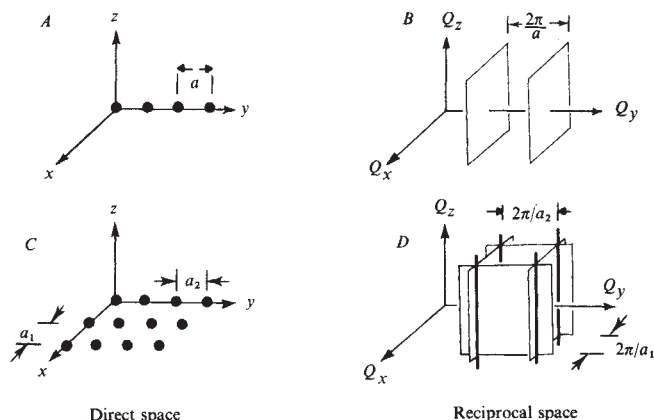


Fig. 2 Atomic arrays in direct space (A, C) and their associated diffraction patterns in reciprocal space (B, D).

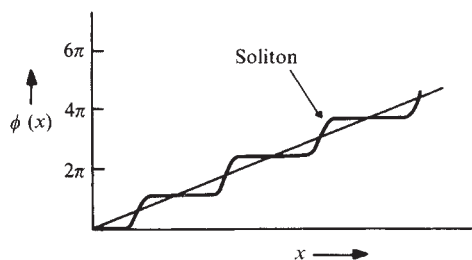


Fig. 4 Variation of the phase $\phi(x)$ (see equation (6)) in an incommensurably modulated structure.

the equations governing the two phenomena are not quite the same. (The relevant equation in this case is known as the Korteweg-de Vries equation.)

Figure 4 shows that the difference between a commensurably distorted phase and an incommensurably distorted one is that the latter contains a finite number of solitons. In many cases, it is found that the intersoliton spacing increases as the temperature of an incommensurably modulated phase is decreased. Finally, the number of solitons may become zero at a transition to a commensurably distorted structure. Such a transition, examples of which have been found in both metallic¹² and insulating systems⁸, has become known as a lock-in transition. At the transition, the periodicity of the distortion 'locks in' to that of the underlying lattice.

Fluctuations

The picture presented so far of an incommensurably distorted structure has been a static one. Nevertheless, at finite temperatures there will be fluctuations of atomic positions about their mean values. As always, these fluctuations can be Fourier analysed in both space and time and each Fourier component represents a normal mode or phonon. In view of the somewhat tangled atomic arrangement which is to be expected in the domain walls (solitons) this Fourier analysis probably involves many coupled terms. It may therefore be more profitable to think about possible fluctuations of the distortion wave (see equation (6)) itself. Two fluctuation modes suggest themselves: phase fluctuations (variations of ϕ with space and time), and amplitude fluctuations (variations of A). The first were originally discussed by Overhauser¹⁸ who suggested the name phason for these modes which consist, in fact, of fluctuations in the positions of the solitons. With lack of originality I (and perhaps others?) adopted the name amplitudon for the second type of mode. Not surprisingly, the ability to name these modes has not greatly accelerated their discovery and, at the time of writing, there has been no unambiguous identification of a phason made in an incommensurably modulated phase.

Phasons have been found¹⁹, however, in the unusual chain compound $\text{Hg}_{3-8}\text{AsF}_6$. In this material, linear chains of mercury cations are imbedded in a tetragonal matrix of AsF_6^- anions. The inter-mercury spacing is incommensurable with the lattice constant of the matrix and the (long wavelength) phasons consist of rigid motion of the chains with respect to the matrix. At sufficiently high temperatures (above 120 K) the mercury chains are uncorrelated and, in a neutron diffraction experiment, produce sheets of scattered intensity similar to those in Fig. 2B. Phasons are observed around these diffuse sheets.

Surfaces

In the above discussion, the word incommensurable referred to the relationship between the periodicities of a basic structure and of the modulation which distorts it. However, the word is also used in a slightly different context, which is well exemplified by the case of a rare gas monolayer on a crystalline substrate. A one-dimensional representation of this system is displayed in Fig. 5. The period which the lattice of gas atoms would adopt in the absence of the substrate is not the same as the period of substrate atoms. The two periodicities are incommensurable.

Since the substrate lattice is fairly rigid the interaction between the substrate and the adsorbed gas atoms tends to modulate the positions of the latter. That is, the substrate tries to impose its periodicity on the adsorbate. The adsorbate is thus analogous to the atomic chain in Fig. 1A, and the substrate is a manifestation of the abstract 'imposed modulation' which was described earlier and which is depicted in Fig. 1D. In view of the similarity between the substrate-adsorbate problem and the modulated-lattice problem, it is not surprising that many features of the solutions of these problems resemble one another. Neutron diffraction studies have been carried out, for example, on a monolayer of argon adsorbed on the surface of graphite²⁰. Since the diffraction pattern of the layer is a set of rods in reciprocal space (Fig. 2C, D), while that of the substrate comprises the familiar Bragg points, the sources of diffraction are distinguishable. In the argon-graphite system the adsorbate periodicity at low temperatures is incommensurable with that of the substrate. In such incommensurable layers a soliton (or domain wall) structure, similar to that discussed for the linear chain, and sketched in Fig. 5, is expected^{21,22}. Transitions between commensurable and incommensurable monolayers have been studied by electron diffraction techniques by Fain^{23,24}, Venables²⁵ and their coworkers.

A phenomenon which occurs in adsorbate-substrate systems and which is related to incommensurability is orientational epitaxy^{26,27}. This uninformative phrase applies to a situation in which the crystallographic (symmetry) axes of an incommensurable adsorbate are rotated slightly with respect to the axes of the substrate. Examples of this effect are found with silver adsorbed on mica²⁸ and with some rare gases (notably Ar^{29})

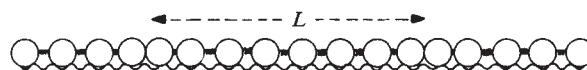


Fig. 5 Artist's view of interacting gas atoms adsorbed on the surface of a crystalline substrate. Two domain walls separated by a distance L are depicted. Between the walls, the gas atoms are in registry with the substrate.

adsorbed on graphite. The effect arises because the interaction energy of the substrate and the (incommensurable) adsorbate depends on the forces, represented by springs in Fig. 5, between the adsorbate atoms. Less energy is needed to produce a shear distortion of the adsorbate than is needed for a compression or contraction of similar magnitude. Thus, the shear is favoured and the adsorbate is rotated with respect to the substrate.

Conclusions

I have attempted to review simply some of the underlying ideas involved in the study of incommensurable systems. Since the subject is still developing, this article must soon be obsolete. Nevertheless, the discovery and study of incommensurably distorted structures is a milestone in the investigation of structural phase transitions and deserves recording. The explicit inclusion of nonlinear effects such as solitons marks a small but significant step away from the traditional treatment of harmonic or weakly anharmonic systems.

Linear atomic chains have been used to illustrate various phenomena for three reasons. First, it is pedagogically simpler. Second, and purely pragmatically, it is only in one dimension that exact solutions to nonlinear problems, such as the Sine-Gordon equation, are available. Third, and of most interest, many of the systems which are incommensurably modulated are one-dimensional in some sense. This applies particularly to the so-called one-dimensional conducting materials, such as TTF-TCNQ, which have received so much publicity during the past 5 years. In these systems, the observation of diffraction patterns similar to that in Fig. 2B is convincing evidence that one-dimensional physics is relevant to the real world.

I thank J. D. Axe, P. C. Hemmer and T. Riste for their critical comments.

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articles

The structure of the radio jets in 3C449

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High resolution observations of 3C449 show the presence of two highly collimated symmetric jets on each side of an unresolved core. The jets show considerable fine structure and polarisation. Measurements of the depolarisation suggest internal densities of $\sim 0.02 \text{ cm}^{-3}$ and flow velocities of $\approx 1,000 \text{ km s}^{-1}$.

INCREASING numbers of low-luminosity radio galaxies have been found to contain highly collimated radio jets which connect the central compact nuclear source to the distant outer lobes. Examples of one-sided jets have been found in radio sources such as NGC6251 (refs 1, 2) and 3C219 (ref. 3) as well as double-sided jets such as found in NGC315 (refs 4, 5) and 3C31 (ref. 6). These jets may be the pathways through which the outer lobes of radio galaxies are continuously supplied by fresh relativistic and thermal material from the central nucleus, although whether the jet is composed of separate components⁷ or a beam⁸ remains controversial. The potential importance of this class of radio source to theories of radio source structure and evolution has led to an extensive programme on the very large array (VLA) to map sources suspected of having radio jets. These observations are being reported elsewhere. Here we present high resolution VLA maps at 20 and 6 cm of the double-sided jet in the radio galaxy 3C449.

3C449 is identified with a cDE4 galaxy⁹, the largest in a group of three (VV 6-49-29)¹⁰. Sandage¹¹ gives the redshift as $z = 0.0181$ and the visual magnitude as $m_V = 13.2$. With $H_0 = 75$ and $q_0 = 0.5$, 3C449 has a luminosity distance of 72 Mpc, and 1 arc s is equivalent to 340 pc linear extent.

3C449 is a member of the open cluster of galaxies Zw2231.2+3732, which itself is less than 30° from the edge of the Perseus supercluster¹², a system having the same redshift as 3C449.

The first interferometric maps showing the structure of 3C449 were made at 408 and 1,407 MHz¹³. More recent maps¹⁴ at three frequencies made with the Westerbork array have clearly delineated the jet-like structure of this source. As the Westerbork observations did not resolve the jet itself, we have made higher resolution maps of the jet with the VLA at 20- and 6-cm wavelengths. Our new observations show that the jet has a highly complex structure.

Observations

The observations were made with the partially completed VLA. Twelve antennas were used, giving spacings from 250 m to 10 km. Eight of the antennas were placed on the south-west arm while the remaining four were on the south-east arm. Data on the array are given in Tables 1 and 2.

The data were phase and amplitude calibrated against 2200+420 (BL Lac) whose position was taken as $\alpha = 22 \text{ h } 00 \text{ min } 39.363 \text{ s}$, $\delta = 42^\circ 02' 08.57''$ (1950) (ref. 15). Three minutes out of every 15 were spent on the calibrator. The flux densities of 2,200+420 were found to be $2.95 \pm 0.01 \text{ Jy}$ and $3.05 \pm 0.03 \text{ Jy}$ at 4,885 MHz and 1,465 MHz respectively. These values were derived from simultaneous observations of 1328+307 (3C286), whose flux density at 4,885 MHz and 1,465 MHz was taken to be 7.41 Jy and 14.44 Jy respectively¹⁶.

Because of the unbalanced distribution of antenna positions on the two arms (the south-west arm having antennas out to 10 km, the south-east arm only to 1.6 km) and the declination of

Table 1 VLA systems parameters for observations of 3C449

Band (cm)	Frequency (MHz)	Bandwidth (MHz)	T_{sys}	Date (1978)	Polarisation
20	1,465	50	60°	29 September	Orthogonal linear
6	4,885	50	50°	1 October	Opposite circular

the source, the u , v plane coverage was non-uniform. In particular, a significant wedge in the u , v plane was not sampled. The resulting synthesised beam contained significant positive and negative side lobes, and the 'CLEAN' algorithm¹⁷ was used to correct for their effects. Map restoration was done with a gaussian beam of the same half-power beamwidth and orientation as the synthesised beam.

As the shortest spacings at the time of our observations were ~ 250 m, some large scale structure is absent from our maps, particularly at 6 cm where the maximum fringe amplitude was $\sim 40\%$ of the total flux. Consequently, no 6-cm maps of structure beyond the jets were attempted. None of our maps have been corrected for the weighting due to the antenna power pattern. Since the FWHM of the primary beam at 6 and 20 cm are 9.0' and 30' respectively, these corrections will be quite small.

Structure of the jets

Figure 1 shows our full resolution 20-cm map with a beam size of 4.8 by 3.4 arc s in position angle (PA) -47° . Perhaps the most striking aspect of this source is the nearly perfect mirror symmetry of the jets and outer lobes about an axis through the central nucleus. Each jet is remarkably straight for some 50 arc s from the nucleus before deviating sharply towards the west, dropping in surface brightness and terminating in large diffuse lobes. As both jets bend in the same direction, rotation effects as a cause of this morphology are ruled out.

These large features continue the symmetry of the source; both bend back towards the east as the distance from the nucleus increases. Although both outer lobes are generally diffuse, several features are of interest. Both lobes are relaxed in appearance, which may suggest that they are in static equilibrium (thermally confined) with their surroundings (as opposed to dynamic or ram-confined equilibrium). In the northern lobe the western edge shows a much sharper rise in brightness than the eastern edge, and there seem to be two parallel ridges of emission orientated along PA $\sim 45^\circ$. In the southern lobe, a ridge of emission is noticeable on the southeastern edge, roughly parallel to another ridge to the north-west. However, there is a significant chance that these ridges are artefacts of the CLEAN algorithm, as the Fourier components representing them will lie in the unsampled segment of the u , v plane. Beyond each lobe,

Table 2 Location of antennas

Antenna no.	VLA station no.	Distance from array centre (km)	Azimuth (deg)
1	W8	0.48	236
2	W32	5.22	236
3	E1	0.04	175
4	W16	1.59	236
6	W24	3.19	236
8	W40	7.66	236
9	W48	10.47	236
11	E8	.48	115
12	E15	1.59	115
13	E12	0.97	115
14	W14	1.26	236
15	W10	0.71	236

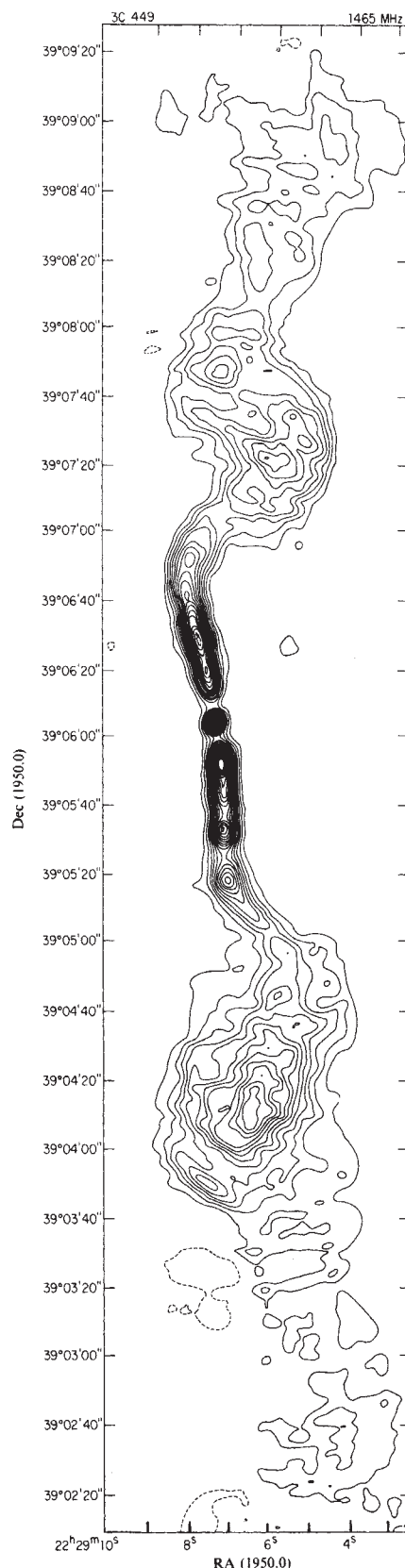


Fig. 1 3C449 at 1,465 MHz as observed by the VLA with a half-power beam of $4.8'' \times 3.4''$ in PA -47° . The contour interval is 5% of the peak brightness starting at +5%. The peak brightness on the map is 22.2 mJy per beam area and is located in the southern jet. The beam response is shown by the unresolved nuclear jet component.

low-brightness emission trails away beyond the edges of the map. A lower resolution map with a beam size of $15''$ was made and showed the diffuse emission extending fully 15.8 arc min or 320 kpc with a slight overall curvature towards the west. The Westerbork 49-cm map of Bystedt and Högbom¹⁴ shows low-brightness radio emission whose extension agrees with that seen in our low resolution map.

Returning to consideration of the morphology of the inner jets, we note: (1) within ~ 10 arc s (3.4 kpc) of the nucleus, both jets are greatly diminished in brightness, although some emission seems still to be present; (2) the southern jet exhibits higher brightness compact condensations. Similar enhancements may also be present in the northern jet although they are not so sharply delineated as the southern ones; (3) the two jets are not quite perfectly opposite each other, but intersect at an angle of $169 \pm 2^\circ$.

A further map, made at $4,885$ MHz, allows us to examine the fine scale structure of the jets. This map, having a resolution of $2.4'' \times 1.0''$ in PA -40° , is shown in Fig. 2. The high brightness condensations noted above at 20 cm are also apparent at 6 cm. The higher resolution shows that each jet is composed of numerous subpeaks or knots superimposed on a more general ridge of emission. Disregarding the inner 10 arc s low-brightness regions of the jets, straight lines drawn along the axes of the jets do not intersect at the nuclear source but at a point some 1.8 arc s north-west in PA -45° . Finally, the jets steadily widen with increasing distance from the nucleus, at a non-uniform rate. The greatest rate of expansion occurs within ~ 10 – 15 arc s from the nucleus where the surface brightness of the jets is minimal. At larger distances the opening angle seems approximately constant. Figure 3 shows the equivalent widths (total area under a cross-section profile divided by maximum height) of the jets measured for our 6 -cm observations and corrected for beam convolution, as a function of distance from the nuclear source. Beyond distances of about 15 arc s from the nucleus, data for both jets are well fitted by a constant opening angle of $\sim 7^\circ$.

The position of the unresolved nuclear source ($< \sim 0.4$ arc s in angular size) was found to be $\alpha = 22$ h 29 min 07.63 s ± 0.01 s, $\delta = 39^\circ 06' 03.5'' \pm 0.1''$. Its flux density was 37 ± 1 mJy and 19 ± 1 mJy at $4,885$ and $1,465$ MHz; thus the nuclear source has a sharply inverted spectrum with $\alpha = -0.55$.

We compared the 6 - and 20 -cm maps to study spectral index variations along the jets; the results are shown in Fig. 4. The computations were made by comparing 6 - and 20 -cm maps made with similar resolutions. Although we are missing considerable flux from the 6 -cm map due to missing short spacings, the jet brightness distribution, being a small scale feature, will be little affected. The missing structure will tend to make the spectrum even flatter than we have measured. In the main lobes, however, so much brightness is absent that no attempt was made to calculate the spectral index for these features. The average spectral index in the jets is 0.65 ± 0.1 in the north jet and 0.5 ± 0.1 ($S_\nu \propto \nu^{-\alpha}$) in the south jet. Some evidence for steepening of the spectrum is noted towards the ends of each jet although, curiously, the spectrum of the southern jet seems to be flattest at a distance of ~ 35 arc s from the nuclear source.

The jets are highly polarised at 6 cm, averaging $\sim 30\%$ along their lengths, although the southern one is somewhat less polarised than the northern one. A plot of the percentage polarisation and measured E-vector PA is shown in Fig. 5. The typical E-vector PA is 160° and changes rather little throughout the length of the jets. Haves¹⁸ found the rotation measure of the integrated polarised flux to be -170 ± 4 rad m^{-2} . If this rotation measure is entirely external and applies to the jets, the intrinsic direction of the projected jet magnetic field is found to be 105° , that is, perpendicular to the jets to within our measurement errors. However, the polarised flux from the outer lobes must make a strong contribution to the total polarised flux and we must be wary of how representative Haves' rotation measure is in the vicinity of the jets, although a large negative rotation measure is characteristic of the galactic coordinates of 3C449

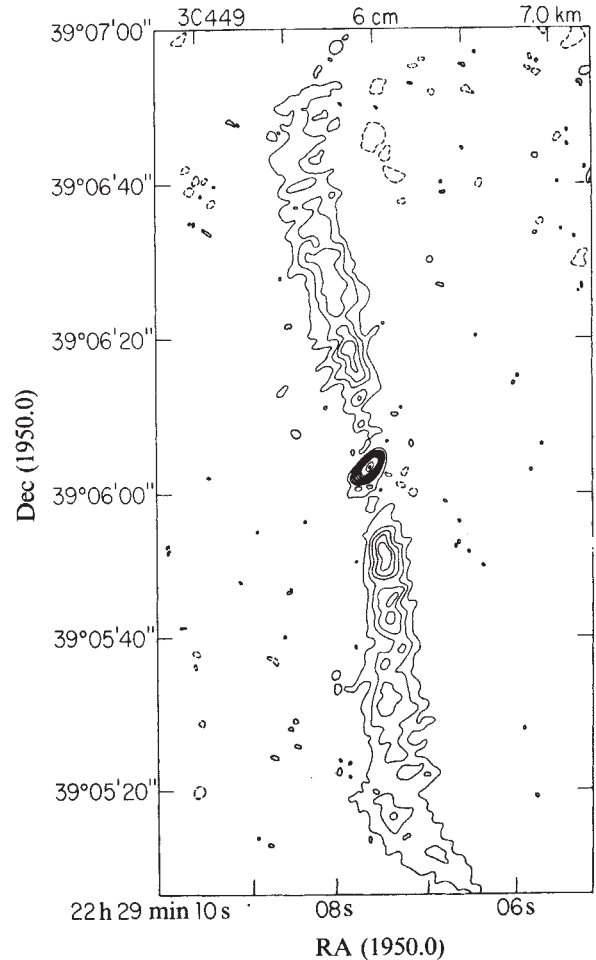


Fig. 2 The high resolution, $4,885$ MHz map of the jets in 3C449. The synthesised beam is $2.4'' \times 1.0''$ in PA -40° . The contour interval is 1.5% of the peak brightness, starting at 1.5% , and the brightest contour is at 13.5% of the peak brightness, which is 37 mJy per beam area. The beam is shown by the nucleus, a 50% contour has been added to delineate the half-power beamwidth.

(ref. 19) ($l = 95^\circ$, $b = -16^\circ$). If our derived orientation of the projected magnetic field is correct, then the most likely intrinsic structure is that of a circumferential field.

Discussion

We calculated equipartition parameters for the outer lobes and the jets using the standard formulae²⁰ but modified to derive the parameters directly from the measured integrated (over frequency) surface brightness, I , and path length through the emitting volume, s , rather than radio luminosity and source volume. The resulting field strength is given by

$$H_{\min} = \left[6\pi K \frac{I}{s} \frac{(1+k)}{|\sin \theta|^{3/2}} \right]^{2/7} \quad (1)$$

and the total energy density in particles and fields is

$$\mu_{\min} = \frac{1}{24\pi} \left[6\pi K \frac{I}{s} \frac{(1+k)}{|\sin \theta|^{3/2}} \right]^{4/7} \quad (2)$$

where k is the ratio of the energy in protons to electrons, and K is equal to $[c_2 c_{12}(\gamma)/c_1 c_3 c_8(\gamma)]$. The c -factors are defined and tabulated by Pacholczyk²⁰ and are functions of γ , the energy index of the assumed power law distribution of relativistic electrons. γ is related to the spectral index by $\gamma = 2\alpha + 1$. The angle θ is measured between the line of sight and the direction of the (assumed uniform) magnetic field.

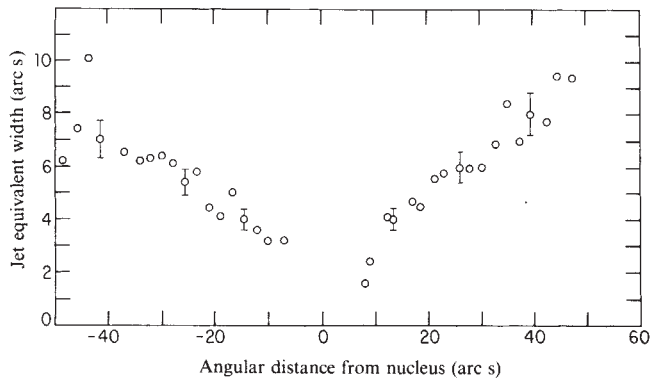


Fig. 3 The equivalent width of the jets found by planimetry of the 6-cm map shown in Fig. 3. The error bars shown are estimated from the noisiness of the corresponding profiles.

Our equations (1) and (2), combined with our 6- and 20-cm measurements of the intensity, give the magnetic field and total energy densities displayed in Table 3. The following assumptions have been made: (1) cylindrical geometry with the jet axis perpendicular to the line of sight, so that the path length through any feature is equal to the observed width of that feature. For the jets, we have taken the path length to be double the equivalent 6-cm width. This is appropriate for brightness distributions which are highly peaked at the centre (and is exactly true for a triangular brightness distribution). Our 6-cm profiles of the jet clearly show the brightness is in fact strongly peaked at the centre, and is not at all limb brightened, as may be expected for emission from a cylindrical sheath surrounding the jet. However, note that this observation is not conclusive evidence for emission from a filled cylinder, as emission from a circumferential magnetic field geometry (the configuration implied by the polarisation measurements) will appear centre brightened for virtually any distribution of the emitting gas due to the highly directive emissivity of synchrotron radiation. (2) The magnetic field lines are perpendicular to the line of sight through the centre of the feature, so that $\sin \theta = 1$. Note that if the field is purely circumferential, this requirement is automatically satisfied whatever the orientation of the jet may be, whereas if the magnetic field is longitudinal, our assumption requires the jet axis to be perpendicular to the line of sight. (3) The spectral index in the jet is 0.5, and 0.7 in the lobes. (4) The integration over frequency is from 10 MHz to 10 GHz. (5) Equal energies in relativistic electrons and protons ($k = 1$).

Note that the equipartition values of the internal energy density in the lobes ($\sim 5 \times 10^{-12} \text{ erg cm}^{-3}$) is approximately equal to the thermal particle energy density (nkT) in the intracluster medium of x-ray clusters of galaxies. Since 3C449 is located in a cluster of galaxies (albeit a poor one) and since HEAO 1 data²¹ demonstrate that poor clusters can also contain X-ray emitting gas, it seems that the radio lobes may be statically confined by external thermal pressure. The diffuse trailing emission is then interpreted as gas rising out of the intracluster medium through buoyancy forces, the overall symmetry of the large scale features being due to the central location of 3C449 in the cluster.

Table 3 shows that in both the northern and southern jets the quantity BR is approximately constant, where B is the magnetic field strength and R is the path length through the jet. This result implies that $B \propto r^{-1}$, where r is the cross-sectional radius of the jet, as suggested by Blandford and Rees⁸ for a jet with a circumferential field. A similar result was found by Readhead *et al.*² for the jet in NGC6251, and it would be interesting to measure the orientation of the magnetic field in that source.

We now consider depolarisation within the jets. They have a typical degree of polarisation of $\sim 30\%$ and, since the electric vectors are quite uniform, it seems that beam depolarisation is unlikely at 6 cm. Thus we must inquire whether the observed degree of polarisation indicates the intrinsic degree of ordering

of the magnetic field or whether significant depolarisation due to thermal matter in the jet has already occurred at 6 cm. We first consider the latter possibility and note that Westerbork 21-cm observations of the jet in NGC315 (ref. 5) show that jet to have an intrinsic degree of polarisation of up to 60%. Adopting this value as a possible intrinsic polarisation for 3C449 and using the simple slab depolarisation model of Burn²² we find a rotation measure internal to the jet of $\sim |500| \text{ rad m}^{-2}$. If the path length is $\sim 3.4 \text{ kpc}$ then $nH_z \sim 1.8 \times 10^{-7} \text{ cm}^{-3} \text{ G}$, where n is the thermal matter density and H_z is the line of sight component of the magnetic field. $H_z \leq \sim 10^{-5} \text{ G}$ (Table 3) so $n \geq \sim 0.02 \text{ cm}^{-3}$. An internal rotation measure of $\sim |500| \text{ rad m}^{-2}$ would imply that the average degree of polarisation in the jet at 21-cm wavelength is only $\sim 4\%$ if the slab model means anything. The unpublished 21-cm Westerbork observations of Bystedt and Högbom show the jet polarisation to be $\sim 4\%$, a value in agreement with what we find.

The low 20-cm polarisation may be due to differential galactic Faraday rotation across the 20-cm WSRT beam. This would require a gradient in the rotation measure of $\sim 50 \text{ rad m}^{-2}$ over an angular scale of $35''$. This is an extremely large gradient, and although no information on rotation measure gradients over this angular scale is available, we regard this explanation for the low 20-cm polarisation as unlikely. The issue will only be settled by high resolution 20-cm polarisation measurements of the jet—planned for late 1979.

We consider now the possibility that the 6-cm 30% polarisation of the jets represents the intrinsic degree of polarisation. In that case a considerable amount of energy is stored in a random magnetic field. Since the 21-cm degree of polarisation is $\sim 4\%$ (ref. 14) there is strong depolarisation by 21 cm. In fact, 21-cm polarisation divided by intrinsic polarisation equals 0.13 and, with this large depolarisation, the rotation measure could be any of several values as we could have gone through the first minimum of the $\sin \chi / \chi$ depolarisation curve appropriate for a simple slab. However, the minimum acceptable rotation measure is $\sim |75| \text{ rad m}^{-2}$, which implies $nH_z \approx 3.4 \times 10^{-8} \text{ G cm}^{-3}$. The uniform component of magnetic field is, in this case, $\leq \sim 5 \times 10^{-6} \text{ G}$ so $n \geq \sim 0.004 \text{ cm}^{-3}$. This limit for the thermal density is not greatly different from that found for the previous case, and since $|75| \text{ rad m}^{-2}$ is a minimum rotation measure, we adopt an internal thermal density of 0.02 cm^{-3} . This value is in good agreement with the density derived for the jet in 3C31 from dynamical arguments²³. Since the 3C449 jets seem to have a large internal rotation measure, the integrated rotation measure¹⁸ may not affect the direction of the jets and our derived magnetic field direction cannot yet be considered definitive.

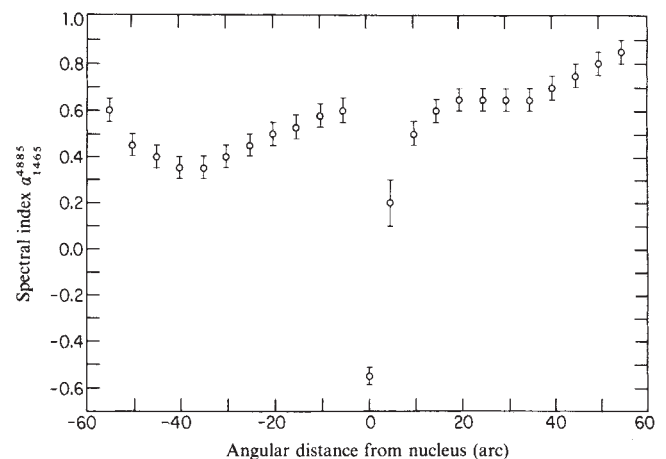


Fig. 4 The measured spectral index of the jets, defined through $S_\nu \propto \nu^{-\alpha}$. The error bars are estimated from the noise in the data, and do not include the possible effects of unmeasured brightness components. The effect of such components will be to lower generally the measured values of α .

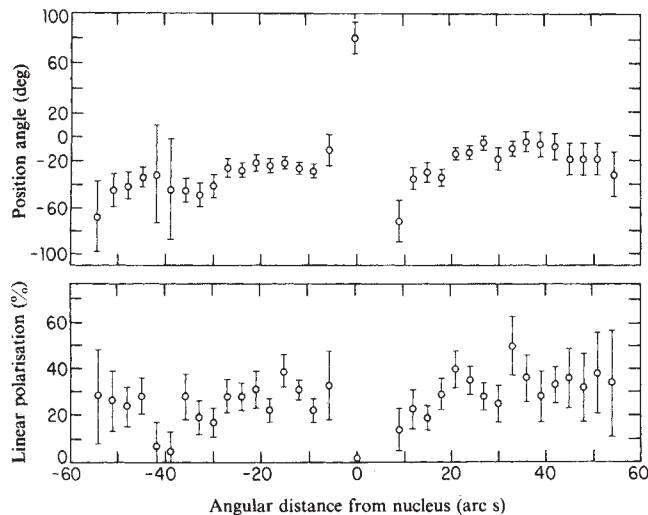


Fig. 5 The percentage and position angle of linear polarisation in the centre of the jets in 3C449 at 6 cm. In this case, the errors have been calculated using the law of propagation of errors from the observed errors in the Stokes parameters I , R , and U . The systematic error due to the polarised side lobes of the VLA antennas has not been included; however, these errors are negligible as they are $<1\%$ within the angular extent of the jets.

At distances greater than ~ 10 arc s from the nucleus the jets expand with a uniform opening angle of $\sim 7^\circ$. If the jet is free (not confined by a surrounding medium), the transverse expansion will be at approximately the generalised sound speed, $(\mu/\rho)^{1/2}$, where μ is the internal energy density and ρ is the mass density. A lower limit to the internal energy density in the jet is given by the energy density in relativistic particles and fields, $\sim 7 \times 10^{-1} \text{ erg cm}^{-3}$ (Table 3) while we estimated ρ to be $\sim 3.3 \times 10^{-26} \text{ g cm}^{-3}$ from the depolarisation calculations. The resulting sound speed is $\sim 150 \text{ km s}^{-1}$. From the opening angle, we find a Mach number of 8 ($= \cotan 7^\circ$) which implies a flow speed of $\sim 1,200 \text{ km s}^{-1}$. This velocity compares favourably with the flow velocity in the jet of 3C31 derived on the basis of dynamical arguments²³. Furthermore, this value is comparable to that suggested²⁴ for the velocity at which material is ejected from the nucleus of the head-tail galaxy NGC1265.

Our estimate of the flow speed depends on the assumption that the transverse expansion is at the internal sound speed, C_s . We note that in an expanding jet, C_s will decrease with increasing distance from the nucleus as the flow becomes more ordered. Thus the lateral expansion must be at least C_s , and so our derived flow speed is a minimum. Furthermore, if the jet is thermally confined, the flow speed will be higher than for a free jet of the same width.

The decline of the internal sound speed with distance from the nucleus may be expected to cause a decrease in the jet opening angle with distance. Note that such a curvature may be occurring in the inner $10''$ of each jet, as seen in Fig. 3.

Further evidence for flow speeds of the order we have estimated is given by the changes in the spectral indices along the jets (Fig. 4). The steepening of the spectrum seen at distances greater than ~ 35 arc s from the nucleus indicates (from synchrotron ageing arguments)²⁰ that the age of the jet at this point is $\sim 1.1 \times 10^7 \text{ yr}$. This distance then translates into a velocity of 800 km s^{-1} . Considering the uncertainties in these types of calculations, we feel that this value is in good agreement with our previous estimates.

The flow speed of $\sim 1,000 \text{ km s}^{-1}$, combined with a mass density of $3.3 \times 10^{-26} \text{ g cm}^{-3}$ gives a mass flow of $2 \times 10^{26} \text{ g s}^{-1}$, corresponding to $3 M_\odot \text{ yr}^{-1}$. This mass flow corresponds to an energy flow of $1.5 \times 10^{42} \text{ erg s}^{-1}$, a quantity which exceeds the directly visible energy flow in relativistic material by a factor of ~ 20 , and which may be a significant source of energy available for production of relativistic matter in the outer lobes.

We have previously noted that each jet bends towards the east before terminating in large, diffuse lobes, and that these parallel bends rule out rotation of the nuclear source. It may seem attractive to invoke the radio-trail hypothesis²⁵ to explain these bends, but because the lobes bend back towards the axis defined by the jets, this hypothesis seems unlikely. Rather, the similarity of the structure of 3C449 to that of 3C31 (ref. 6) suggests that the gravitational encounter hypothesis of Blandford and Icke²³ would be more successful in explaining the bends in 3C449. In this hypothesis, Blandford and Icke have had at least partial success in explaining the symmetry of 3C31 by invoking a gravitational interaction between 3C31 and a nearby companion. Since 3C449 has two companions²⁶ located within $100''$, the symmetry in this source may be due to a similar interaction between one or both.

Beyond the large lobes, the emission is seen to trail away with a small but distinct curvature towards the west. This structure is reminiscent of the general structure of radio tail galaxies, and it is worth noting other similarities between 3C449 and radio tail galaxies such as 3C83.1B (NGC1265). The luminosity of 3C449 is typical of radio tail galaxies, and the observation that the compact structure of 3C449 is located close to the optical galaxy is also similar to NGC1265. Also, our derived flow speed ($\sim 1,000 \text{ km s}^{-1}$), mass flow rate ($\sim 3 M_\odot \text{ yr}^{-1}$) and ratio of bulk kinetic to relativistic energy (20:1) agree with theoretical values taken from the fit of the plasmoid model²⁷ of radio tail galaxies.

There is increasing observational evidence that gaps near the nuclear source seem to be a common property in radio jets, having been observed in several radio sources (for example, NGC 1265²⁸, NGC 315⁵ and 3C219³). The typical physical separation is usually several kpc. At present we can only suggest some explanations. The gaps may represent the intervals between major outbursts. The typical separation between major peaks in the 3C449 jets is 3.4 kpc, corresponding to $\sim 3 \times 10^6 \text{ yr}$ with our previously estimated flow speeds. This time interval between major outbursts is in good agreement with that calculated²⁴ for NGC1265. Another suggestion is that the material flowing from the nucleus is initially very highly collimated so that synchrotron radiation is inhibited by a small pitch angle between the particles and magnetic field. This suggestion implies that close to the nucleus the magnetic field should be parallel to the jet. VLA observations of 3C31 and NGC315²⁹ suggest that this configuration may indeed prevail. Alternatively the magnetic field could be much lower than the equipartition value (a decrease by a factor of 20 would suffice to produce the observed drop in brightness). If either of the last two suggestions holds true, then some agency, possibly turbulence, is required to randomise the particle pitch angles or amplify the magnetic field strength, or both, by the time the material has travelled several kpc from the nucleus.

This latter suggestion may relate to the changes in spectral index and 6-cm degree of polarisation along the jets (Figs 4 and 5). In the northern jet, the spectrum continually steepens as the distance from the nucleus increases; at the same time, the degree of polarisation steadily increases, which would indicate an increasingly ordered magnetic field. The spectrum of the southern jet flattens over the distance $0\text{--}35''$ from the nucleus while the degree of polarisation apparently decreases over this

Table 3 Equipartition values of magnetic field and total energy density at selected points in 3C449

Site	Emissivity* I/S ($\text{erg s}^{-1} \text{ cm}^{-3} \text{ sr}^{-1}$)	$H_{\min}$ (μG)	$\mu_{\min}$ (erg cm^{-3})
Southern Lobe	4.5×10^{-29}	7.3	4.9×10^{-12}
Northern Lobe	3.8×10^{-29}	7.0	4.5×10^{-12}
Southern Jet $40''$ S	2.1×10^{-28}	8.1	3.9×10^{-12}
Southern Jet $15''$ S	6.8×10^{-28}	11.2	1.2×10^{-11}
Northern Jet $15''$ N	5.6×10^{-28}	10.6	1.0×10^{-11}
Northern Jet $35''$ N	1.6×10^{-28}	7.4	5.1×10^{-12}

* From 10 MHz to 10 GHz assuming a power law spectrum of index 0.5 for the jets and 0.7 for the lobes.

distance, although the errors are large. Thus the magnetic field in the southern jet apparently becomes increasingly disordered. This disordering might be due to turbulence which then might also be responsible for transferring energy to the relativistic electrons²⁷ and thus for flattening the observed radio spectrum.

Conclusions

The low-luminosity radio galaxy 3C449 exhibits great variation of structure. Two compact, well collimated, and nearly symmetric jets are found to issue from an unresolved core which is optically thick at 6 cm and coincident with a cDE4 optical galaxy. The radio jets are not smooth in appearance, showing a lumpy and somewhat turbulent structure. The jets are not seen within 10'' (~3 kpc) of the central core, but appear to 'turn-on' at this distance. Each jet widens steadily beyond the turn-on point, and gradually diminishes in brightness until each makes a sharp bend to the west and terminates in large, diffuse blobs with very relaxed appearance. Trailing emission up to ~8' from the centre is detected.

Received 30 April; accepted 22 August 1979.

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The spectrum of each jet is flatter than normal, with the spectral index averaging near 0.6. Little trend in the index is seen, although the southern jet may flatten slightly towards the end. Both jets are highly polarised, with percentage linear polarisation varying generally from 20 to 40%. The position angle of the polarisation is nearly constant throughout each jet. If all, or most, of the Faraday rotation is external, then the inferred jet magnetic field is circumferential. However, a significant portion of the rotation measure may be due to internal thermal matter as WSRT observations of the jet show low polarisation. If this depolarisation is due to internal Faraday rotation, the inferred electron density is $\sim 0.02 \text{ cm}^{-3}$. Combining this mass density with minimum energy densities derived from standard equipartition arguments, a minimum flow speed of $\sim 1,000 \text{ km s}^{-1}$ for a free jet is found.

We thank J. Bystedt and J. Högbom for permission to use their data before publication, and J. O. Burns for useful comments. A.G.W. acknowledges support from the NSF under grant AST 77-22906.

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Jupiter's rings

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Voyager 2 observations of the jovian ring system discovered by Voyager 1 are presented. The rings were observed both above and below the jovian equatorial plane and at extremely low and high phase angles. This ring system seems to represent a steady state configuration for small particles that are slowly moving in toward Jupiter. It must be resupplied from sources well outside Roche's limit.

JUPITER'S ring system was discovered on 4 March 1979 by Voyager 1 as a result of a deliberate search as the spacecraft passed through the equatorial plane of the planet¹. Having established the existence and outer dimension of a jovian ring system, Smith *et al.* worked with the Voyager engineers to develop new imaging sequences for the Voyager 2 encounter with Jupiter during early July. The major goal of these sequences was to make observations slightly out of the ring plane, in order to establish the number of ring components present, their dimensions and relative brightness. Several filters were used to establish the colour of the ring particles. A summary of Voyager's jovian ring data set is given in Table 1. Figure 1 illustrates the geometry and times at which Voyager 2 data were taken with respect to the flyby trajectory.

Data description

The first set of newly sequenced images was obtained on 8 July at an inclination of 2.5° to the ring plane on the inward leg of the trajectory. These data were the first to reveal both sides of the ring as seen in Fig. 2. This view confirmed that the jovian ring was very faint and showed no obvious gaps. The brightest segment of the ring in Fig. 2 was found to be $\sim 3 \times 10^{-5}$ of the mean brightness of Jupiter at zero degrees phase angle. These data were smeared by relative spacecraft motion (apparent in the star trails recorded in 96-s exposures).

The next set of images was obtained 14.5 h later as the spacecraft passed through the planet's equatorial plane. Because of image motion, these pictures did not immediately add to the information already available from Voyager 1, but they confirmed the existence of a sharp outer edge for the ring system (Fig. 3). This boundary has been remeasured on the Voyager 1 image and has a value $1.8 R_J$ (128,500 km from the centre of Jupiter).

All of these observations (including the Voyager 1 picture) were obtained at rather small phase angles; the rings were visible because of light scattered back towards the camera. At the time of the Voyager 2 encounter the Sun was in Jupiter's equatorial plane with about two thirds of the solar disk below (south of) the ring plane as seen from Jupiter.

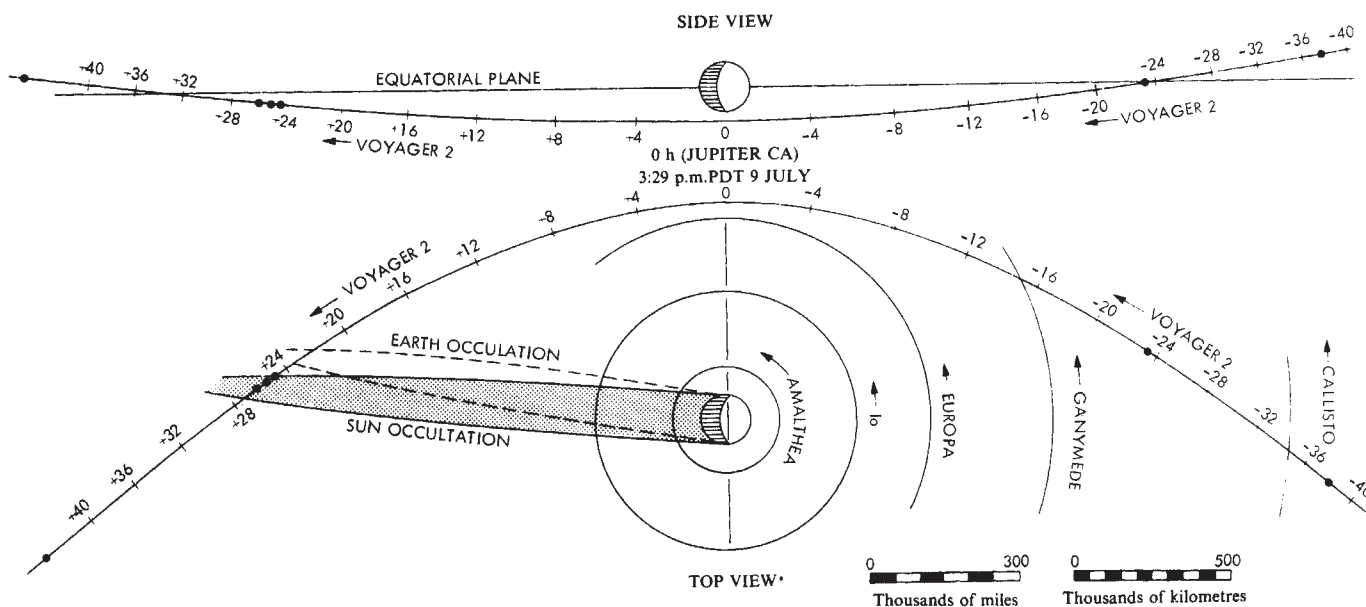


Fig. 1 The Voyager 2 spacecraft's Jupiter flyby trajectory is shown in two views. Each of the sets of pictures targeted for the rings is shown by the symbol ●. The time of closest approach for Jupiter was 11.21 UT 9 July 1979 (4.21 p.m. PDT 9 July). The range at closest approach was 10 R_J .

Twenty-six hours after closest approach to Jupiter, the spacecraft was in Jupiter's shadow approximately two degrees below the ring plane. The pictures taken in these illumination conditions at very high phase angles, resulted in the spectacular images shown in Figs 3, 4, 5, 6 and 7. Close inspection of the complete set of pictures indicates that there are at least four distinct components in the jovian ring system:

- a narrow bright segment about 800 ± 100 km wide, inner edge $1.7 R_J$;
- a fainter segment about 6,000 km wide, inner edge $1.68 R_J$;
- A very faint inner 'ring' that appears to be a continuous sheet of dispersed material probably extending all the way to the planet;
- an even fainter outer edge of small particles that extend to $1.8 R_J$ and slightly beyond.

Each of these components is designated in the intensity plot of Fig. 8. The intensity plot was taken normal to both sides of the ring halfway between the visible end of the ring and Jupiter.

Note that the jovian ring system is thus closer to its parent planet than either of the rings of Saturn (outer edge of ring A = $2.28 R_S$) or the rings of Uranus (outer edge of ring ϵ = $2.1 R_U$). On the other hand, the width of the jovian ring is intermediate between the extremely narrow rings of Uranus (<100 km) (ref. 2) and the broader rings of Saturn (17,000–25,000 km).

Steady-state rings

We favour the following hypothesis for the origin of the rings and their maintenance: namely, that the rings seem to represent a steady-state configuration for small particles moving in towards the planet in orbits near the equatorial plane. The particles are supplied from sources outside the Roche limit which could include cometary and meteoritic debris, impact ejecta from the inner satellites, and volcanic ejecta from Io. Evidence of loss from the ring is apparent from the observed distribution of particles inside the bright segment, ring (c). Among the loss mechanisms to be considered we note the Poynting–Robertson effect both from direct sunlight and that reflected by Jupiter, but interactions with the jovian magnetic field and the environment of charged particles are probably more important. Braking by the jovian magnetic field (which is moving more slowly than particles in the ring) will cause charged grains to move inwards. The high energy ions in the jovian magnetosphere will sputter material from them, causing the particles to get smaller and thus enhance the previously mentioned braking mechanisms.

Preliminary analysis

A rough estimate of the mean particle size in the jovian rings can be obtained from the phase function manifested in the strong forward scattering. In the narrow angle frames, it is evident that the arm of the rings closer to the spacecraft is less bright than the farther one. This is shown in Fig. 8 where a plot of relative intensity is shown for both the near and far sides. This set of data represents a difference in scattering angle of $\sim 0.3^\circ$. Inspection of intensity contours for single particle scattering³ suggests that for this condition to exist, the size parameter $X = 2\pi a/\lambda$ must be about 50. Adopting $\lambda = 0.497 \mu\text{m}$ as the effective wavelength of the narrow angle camera with the clear filter (a clear quartz substrate) we find that the mean particle radius is in the range of $4 \mu\text{m}$. This estimate is also supported by comparing the brightness of the scattering at this high phase angle with that obtained near opposition. The Voyager 2 data show that the rings are approximately 20 times brighter in the diffraction lobe than in backscattering. (Note: the actual maximum value of this difference must be greater still.)

It is also evident from colorimetry based on comparisons of images taken through violet, orange and green filters that the light diffracted towards the spacecraft by the ring particles at phase angles near 180° is distinctly reddish compared with sunlight (the ratio of orange to violet is 1.7–1). This suggests either blue absorption by the particles or wavelength-dependent forward scattering (diffraction). At this stage, it is not possible to determine which of the two causes is dominant. Note that low

Table 1 Voyager 1 and 2 observations of jovian ring system

Date/time UT	No. of pictures	Filter	Camera*	Exposure time (s)	Angle†
4 March/19.38	1	Clear	NA	672	0°
8 July/8.39	2	Clear	NA	96	$+2.5^\circ$
8 July/23.04	6	Clear	NA	96	0°
	1	Violet	NA	96	
	1	Green	NA	96	
	1	Orange	NA	96	
	1	Clear	WA	15	
10 July/00.48	2	Orange	WA	96	-2.0°
	2	Violet	WA	96	
	7	Clear	NA	96	
	1	Clear	WA	15	

* NA, narrow angle; WA, wide angle.

† The angle of the spacecraft above or below the jovian ring plane.



Fig. 2 Both sides of the jovian rings are apparent in this two picture mosaic returned by Voyager 2. These data were taken at a range of 2×10^6 km when the spacecraft was approximately 2.5° above the ring plane. The data were submitted to an extreme contrast enhancement to bring out this faint signal. The blotchy areas around and between the rings are artefacts of this extreme enhancement.

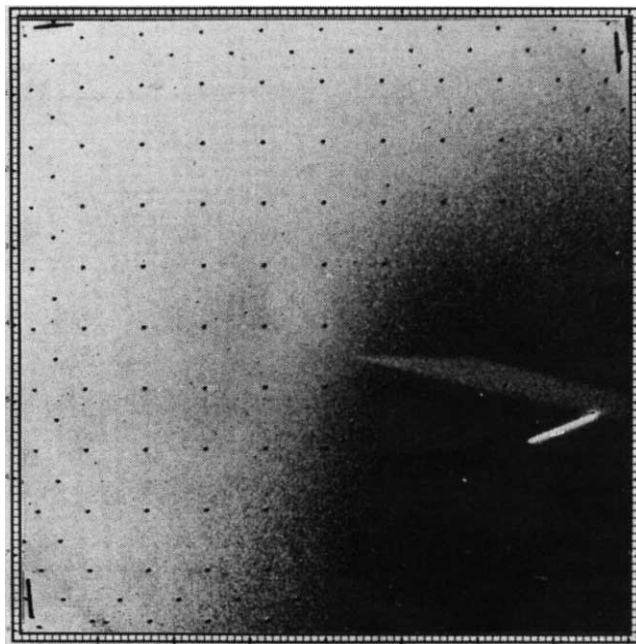


Fig. 3 This Voyager 2 picture caught the sharp outer edge of the ring as the spacecraft passed through the ring plane at a range of 1.4×10^6 km from Jupiter. The bright star trail shows the relative spacecraft motion which smeared the picture in this 96-s exposure through the clear filter. This was one of a set of nine pictures targeted at this time.

reflectivity of blue light is consistent with the low blue albedos of all five large jovian satellites (especially JV and JI) and most asteroids and meteorites^{1,4,5}. The colour by itself, however, is not diagnostic of composition.

Resonance commensurabilities

The existence of a well-defined outer boundary for the rings suggests a decrease in the drag force acting on inward evolving particles moving into this general region. The fact that the brightest segment of the ring (a) is at this outer boundary simply reinforces this impression. There are two near commensurabilities between the period of particles at the ring's outer edge and fractional periods of jovian satellites:

$$\frac{3}{5}P_{\text{Amalthea}} \text{ and } \frac{1}{6}P_{\text{Io}} \text{ (in days: } P_{(a)} = 0.291, \frac{3}{5}P_v = 0.299, \frac{1}{6}P_I = 0.295)$$

These near commensurabilities do not seem particularly compelling, as the mass of Amalthea is very small ($\sim 10^{21}$ g if $\rho = 3.4$) and $1/6$ is a small fraction of Io's period. For comparison, the outer edge of Saturn's A ring falls close to a $2/3$ resonance with Mimas, while particles in Cassini's Division would have approximately half the period of Mimas, but even there the agreement is not perfect⁶. Note that if Amalthea were considered to have a role equivalent to Mimas for the Jupiter system, the entire (a) + (b) complex falls well inside the boundaries set by the $1/2$ and $2/3$ resonances. It may be that in the Jupiter system the satellite resonances only serve to modulate other effects, for example, a balance between electrostatic and gravitational forces.

Relation to Roche limit

In any case, the rings are well inside the classical Roche limit for the breakup of a liquid satellite ($2.44 R_J$) and also within the limit set by the mutual gravitational attraction between two particles with mean densities differing from that of Jupiter:

$$r = R_J \left(16 \frac{\rho_J}{\rho_p} \right)^{1/3}$$

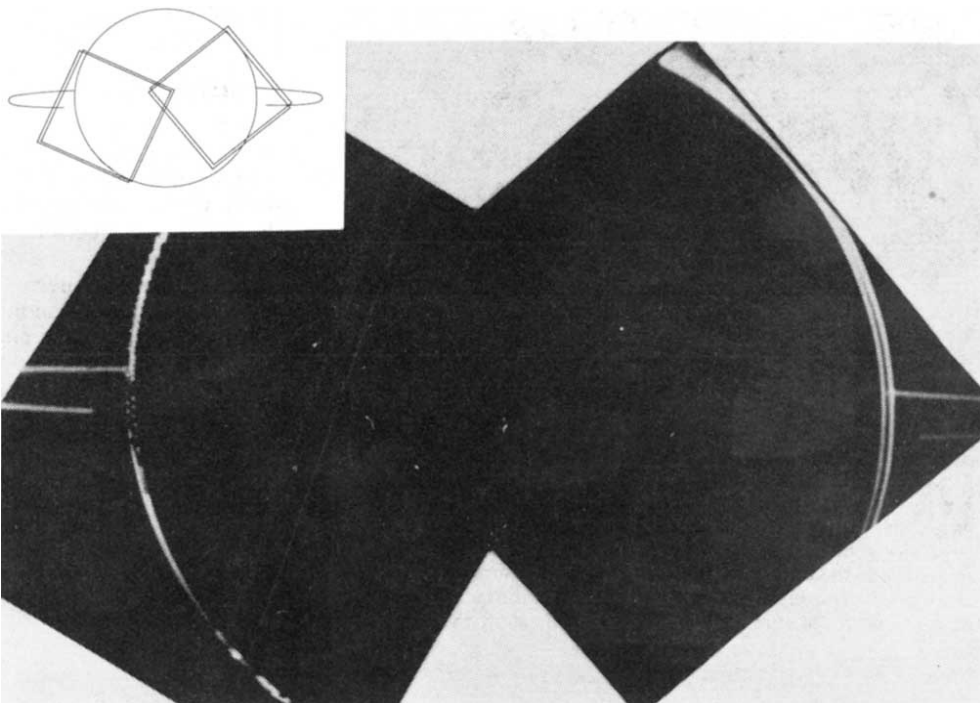


Fig. 4 This mosaic was taken while the spacecraft was in Jupiter's shadow at a range of 1.45×10^6 km. These pictures were taken with Voyager 2 spacecraft's wide angle cameras through the violet filter. The limb of Jupiter and the rings are clearly revealed in these high phase angle pictures as a result of forward scattering from a haze layer high in Jupiter's atmosphere.

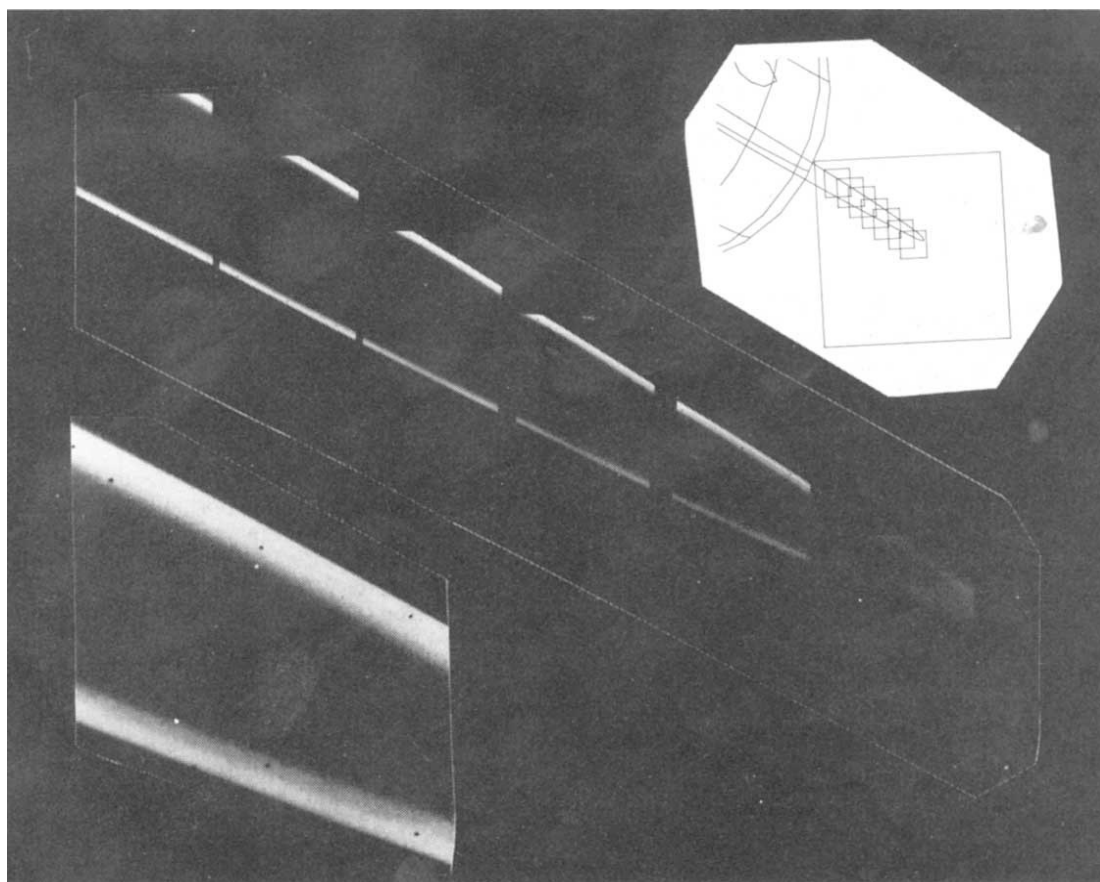


Fig. 5 This six picture narrow angle sequence was taken at high phase angles while the Voyager 2 spacecraft was in Jupiter's shadow. The line drawing in the upper corner illustrates the targeted fields of view predicted for this picture-taking sequence. These data were edited onboard the spacecraft to preserve the signal-to-noise ratio required to send them back in real time (which accounts for their rectangular shape and the loss of overlap). The picture on the lower left has been enlarged to illustrate the presence of material between the bright arms which forms a continuous sheet that may extend all the way to the planet. The star trail shown gives a history of the smear during this 96-s exposure.

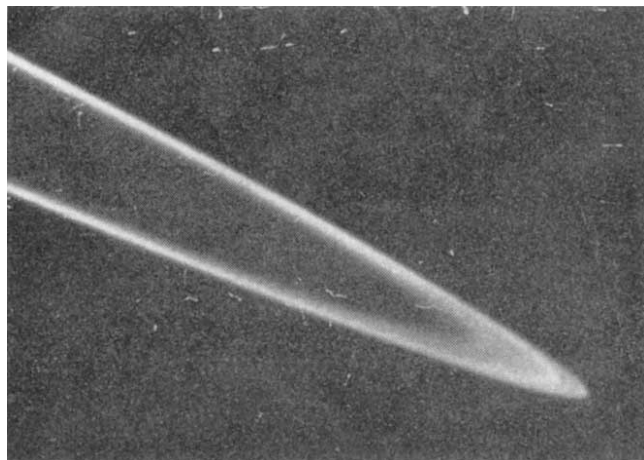


Fig. 6 This wide angle picture was taken at the end of the narrow angle picture sequence in Fig. 5. It shows the bright, sharp outer edge, along with the fainter inner continuous sheet of particles (ring (c)) between the edges. This picture had an exposure time of 15 s and was taken through the clear filter. The spacecraft was at a range of 1.4×10^6 km in Jupiter's shadow about 2° below the plane of the ring.

Adopting $\rho_p \approx 2.6$ (for example, meteoritic material) $r = 2 R_J$. On the other hand, if Amalthea represents a high temperature condensate that continues the density gradient from JIV to JI, it might have a density as high as 3.5, the mean density of Io. Fragments of this satellite in the ring would then lead to $r = 1.81 R_J$, close to the observed outer radius. As the particles in Saturn's rings evidently consist mainly of water ice, this gravitational constraint suggests that one reason Jupiter's rings are so close to the planet may be that the ring particles have a higher mean density than the particles in the rings of Saturn.

The limit for the breakup of a solid body with a diameter larger than 30 km and a very low tensile strength (10^6 dyn cm $^{-2}$) has been calculated by Aggarwal and Oberbeck⁷ to occur between 1.35 and 1.38 R_J , well inside the observed dimensions of the (a) and (a) + (b) components of the ring (1.68–1.77 R_J).

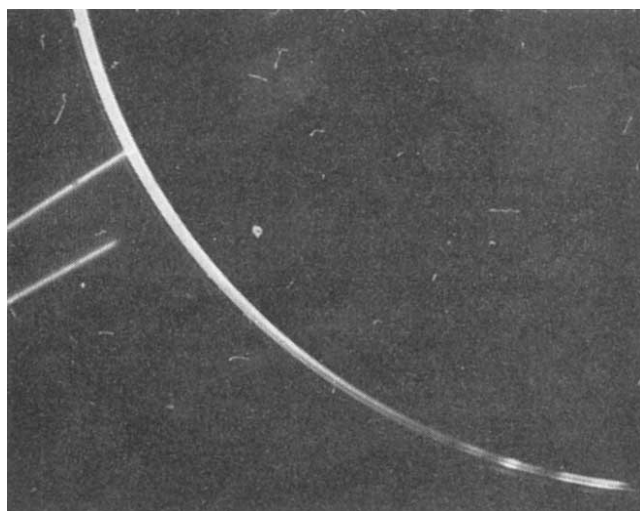


Fig. 7 A black and white reproduction of a colour composite which was made from a violet and orange frame taken with the wide angle camera. These are the same data as shown on the left in Fig. 4. The jovian ring system is two light orange lines protruding from the left along with the very faint orange materials in between them. The edge of the ring closest to the spacecraft has been cut off by Jupiter's shadow. The spacecraft was approximately two degrees below the ring plane. The very colourful multiple images of the Jupiter limb are evidence of image smear during these long exposures (96 s).

Hence, there is no reason to assume we are looking at fragments from a disrupted satellite.

Conclusions

Before this discovery, Pollack⁸ suggested that a ring around Jupiter was unlikely because original particles left over from the planet's accretion would have been quickly lost by gas drag and ice particles would not have condensed late in the accretion ring dissipation stage because Jupiter would have been too hot. Our model for a steady-state ring is entirely consistent with this point of view. It is now necessary to define the sources, sinks and stabilising influences in more detail.

Further study of the pictures of Jupiter's ring should give an improved assessment of the scattering properties and hence average size of the ring particles. The dimensions for the ring

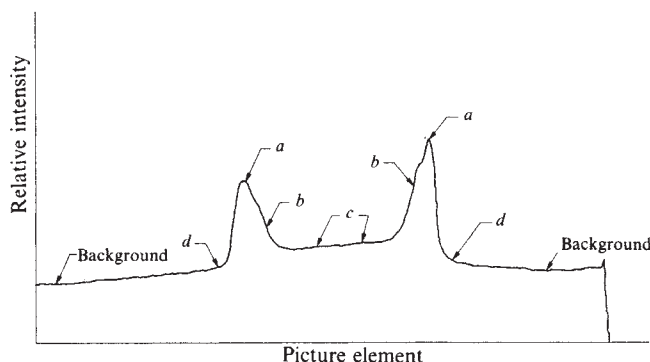


Fig. 8 This plot of relative intensity illustrates the difference in brightness and resolution between the near and far edge of the rings. These data were taken perpendicular to the ring at about $1.4 R_J$. This narrow angle frame is part of the mosaic in Fig. 5 taken through the clear filter. It shows a ratio of ~ 1.43 for the brightest part of each side of the ring plotted in this intensity profile. This plot also clearly shows each of the four components of the jovian ring system. The brightest segment (a) and the fainter and wider inner ring (b) along with the very faint components (c) and (d).

components stated here will be refined and the degree to which the ring approaches a perfect circle will be determined with the help of the images of stars recorded on individual frames. Unfortunately, we will not be able to improve the crude upper limit of 30 km placed on the ring thickness by Voyager 1, as the images obtained at ring-plane crossing with Voyager 2 do not give any better resolution.

We thank the many JPL and NASA personnel who worked on Voyager and made the successful flybys of Jupiter possible. We also thank Dr J. E. Hansen, L. Moriboto and S. Synnott and the navigation team for their support in data reduction calculations. This paper represents the results of one phase of research carried out at the JPL and NASA contract NAS 7-100.

Note added in proof: We have searched carefully for evidence of a shadow from the rings on the surface of the planet, using Voyager 1 images when lighting conditions were optimal. No indication of a shadow was found, even though our resolution was twice the expected shadow width (~ 50 km). Pre-discovery reports of ground-based observations of the shadow—at resolutions more than 150 times worse than that of the spacecraft—are probably incorrect.

Received 22 August; accepted 7 September 1979.

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Directive segregation is the basis of ColE1 plasmid incompatibility

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Incompatibility between ColE1 plasmids in Escherichia coli can be explained by competition for a limited number of replication sites. These sites ensure directive segregation of plasmids to daughter cells on cell division. The number of sites can be more than one only if a linear segregation mechanism is postulated.

Plasmid incompatibility is defined as the failure of two plasmid replicons to be stably maintained in the same bacterial cell. Two general types of models have been postulated to explain plasmid incompatibility. One is derived from the replicon hypothesis of Jacob, Brenner and Cuzin¹. They proposed that each bacterium has a single site at which particular plasmids are replicated. The site acts as a directive segregation mechanism which ensures that each daughter cell receives a copy of the attached plasmid. Incompatibility is due to competition between plasmids belonging to the same compatibility group for this single essential replication site. A second model, proposed by Pritchard and coworkers²⁻³, is based on the observation that an Hfr strain cannot harbour a free F factor. According to this model, incompatibility results indirectly from the action of trans-acting cytoplasmic factors (repressors) whose actual physiological function is to regulate plasmid copy number. When two plasmids produce and are sensitive to the same repressor, there is an increased probability that a daughter cell will not receive both plasmids because the combined copy number of the two plasmids is equal to the normal copy number of each plasmid alone.

Plasmid incompatibility among small non-conjugative plasmids has been investigated^{4,5}, and Warren and Sherratt⁶ have recently demonstrated that any two plasmids derived from ColE1⁷ replicons are incompatible. They found that smaller ColE1 derivatives displaced larger ones and argued that this was consistent with a replication repressor model for plasmid incompatibility. The conclusion was based on the assumption that smaller plasmids have less affinity for the postulated replication repressor than larger plasmids. There is now evidence for the existence of replication repressors⁸. These experiments indicated that pSC101⁹ copy number is controlled by a trans-acting factor.

In the experiments reported here, we have used two families of multicopy plasmids to investigate further the mechanism of plasmid incompatibility. One family is characterised by ColE1 and the other by RSF1030¹⁰. Our results can be summarised as follows: (1) RSF1030 and its derivatives were compatible with ColE1 and its derivatives; (2) when two ColE1 derivatives were used to simultaneously transform *recA*⁻ *E. coli* resultant double transformants existed only temporarily; (3) the hybrid RSF1030-ColE1 replicon and a single ColE1 replicon were stably maintained in the same cell; and (4) multimer forms of pMB9¹¹ were incompatible with each other. Our results support

the view that plasmid incompatibility is the consequence of a directive segregation mechanism for plasmid propagation. The phenomenon of incompatibility is not associated with plasmid copy number control and the results are not consistent with a model postulating that replication repressors are the primary cause of plasmid incompatibility.

Co-transformation of *E. coli* by two plasmids as a system for plasmid incompatibility studies

One way to study plasmid incompatibility is to simultaneously introduce a single copy of each of two phenotypically distinguishable, incompatible plasmids into a single *E. coli* cell. Figure 1 demonstrates that significant numbers of double transformants (arising by simultaneous transformation with two incompatible ColE1 plasmids) could be obtained at DNA concentrations at which double transformants were the result of two independent single-hit events by the two incompatible plasmids. The number of cells obtained that were resistant to a single drug was directly proportional to the concentration of DNA used in the transformation and the saturating DNA concentration of 6×10^{-9} M was the same for each plasmid. We have no simple explanation for the observation that the number of transformants obtained at any particular molar concentration of DNA was greater for RSF1030 than for the ColE1 derivatives pML21¹² and pMB9 and in turn was greater for pMB9 and pML21 than for another ColE1 derivative pSF2124¹³.

Formation of double transformants with two compatible plasmids (RSF1030 and pML21 or RSF1030 and pMB9) was also log-linearly related to plasmid DNA concentration. The slope of the line $\log(\text{number of transformants})$ versus $\log(\text{DNA concentration})$ is ~ 2 , as expected for two independent random events. The number of double transformants also plateaued at 6×10^{-9} M DNA. The formation of pMB9-pML21, pMB9-pSF2124 or pML21-pSF2124 double transformants were not log-linear with DNA concentrations. Nevertheless, significant numbers of double transformants were obtained within the range of plasmid DNA concentrations at which double transformants for compatible plasmids arose by two independent events. We have ruled out the possibility that double transformant colonies are a consequence of mutual crossfeeding of plasmid products from separate pMB9 and pML21 transformants. When pML21-containing cells and pMB9-containing cells were patched together at high concentrations on a plate containing tetracycline (Tc) and kanamycin (Km) no growth of the patch was observed.

Genetic instability and plasmid composition of cells of double transformant colonies

A *recA*⁻ strain (HB101) was simultaneously transformed with pMB9 (Tc^r) and pML21 (Km^r) and the cells were plated immediately on Tc+Km media. The colonies that grew on the double selection plates were purified on nonselective medium so

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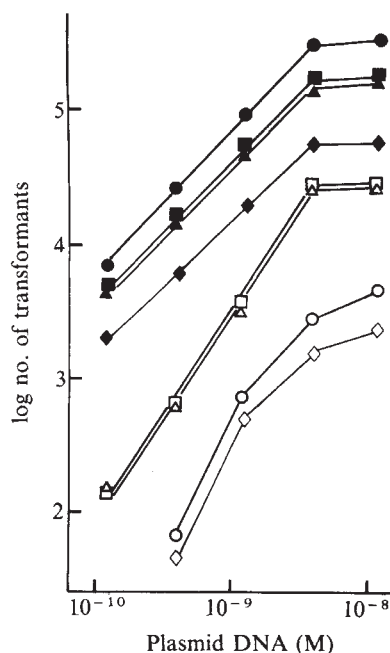


Fig. 1 Frequency of the formation of single and double transformants as a function of plasmid DNA concentration. HB101 ($r_B^- m_B^- F^- pro^- gal^- arg^- recA^-$) (ref. 16) was grown at 37 °C in LB media containing per litre 10 g bacto tryptone (Difco), 5 g yeast extract (Difco) and 5 g CaCl₂ pH 7.2. Cells at mid log growth were collected and resuspended in one half growth volume of chilled 30 mM CaCl₂, incubated at 4 °C for 20 min, pelleted and resuspended in $\frac{1}{50}$ of the growth volume of 30 mM CaCl₂. Approximately 4×10^7 viable CaCl₂ treated cells were incubated at 4 °C with mixtures of pMB9, pML21 and RSF1030 plasmid DNAs or pMB9, pML21 and pSF2124 plasmid DNAs at the indicated DNA concentrations for 60 min. The DNA concentration given is the concentration of each plasmid DNA alone. The cell-plasmid DNA mixtures were incubated at 42 °C for 2 min., diluted 10-fold in LB media, and grown for 20 min. at 37 °C. This time was adequate to give the maximum number of transformants. Aliquots of cells at various dilutions were plated on LB media containing 1.5% Difco agar. To select for the presence of RSF1030 and pSF2124, plates contained 30 μ g ml⁻¹ ampicillin; to select pMB9, plates contained 10 μ g ml⁻¹ tetracycline; and to select pML21, plates contained 20 μ g ml⁻¹ kanamycin. RSF1030 (●), pMB9 (■), pML21 (▲), pSF2124 (◆), RSF1030+pMB9 (□), RSF1030+pML21 (△), pMB9+pML21 (○), and pMB9+pSF2124 (◇). The number of transformants plotted is corrected for a constant viable cell count.

that individual cells from the original colony could be tested for Tc^r, Km^r and Tc^r + Km^r. Each original Tc^rKm^r colony contained cells which were either Tc^r or Km^r. No cells were resistant to both Tc and Km. These results indicated that while primary doubly transformed cells must have contained both plasmids, in further generations individual cells propagated only one of the two plasmids.

Figure 2 shows that the proportion of Tc^r cells in a doubly transformed colony that were Tc^r (contained pMB9) varied between 0 and 100% depending on the Tc concentration. (The Km concentration was 20 μ g ml⁻¹ in all plates and the Tc concentration was 1–10 μ g ml⁻¹). We interpret these results as follows: Primary transformants containing both pMB9 and pML21 synthesise products which confer Tc^r and Km^r, respectively. These products are distributed to daughter cells. Some daughter cells propagate pMB9 and others propagate pML21. The cells propagating pMB9 are Tc^r and, for some period of time, are also Km^r, as they received Km^r-conferring products from the primary transformed cell. Similarly, cells propagating pML21 are Km^r and retain a Tc^r phenotype until the Tc^r-conferring products are diluted out. The number of divisions before the critical dilution occurs when daughter cells become Tc^s will depend on the Tc concentration in the medium. Thus the higher the concentration of Tc, the lower the proportion of cells in the double transformant colony that contain pML21. In

contrast, at very low Tc concentrations, cells propagating pML21 will have a significant growth advantage over those propagating pMB9 as the latter cells must detoxify the Km in the medium with pML21 products which are continually being diluted at each cell division. Thus, this experiment supports the notion that doubly resistant transformant colonies are a mixture of two cell types, each type propagating one of the two plasmids initially entering the primary transformant.

Table 1 Description of plasmids used

Plasmid	Compatibility group	Description	Relevant phenotype	Source or reference
pMB9	ColE1	pMB1 derivative	Tc ^r	11
pML21	ColE1	ColE1 derivative	Km ^r	12
pSF2124	ColE1	ColE1 derivative	Ap ^r	13
RSF1030	RSF1030	Naturally occurring	Ap ^r	10
pJC307	ColE1	Temperature sensitive mutant	will not replicate at 42 °C	15
pHM15	ColE1	pJC307::Tn5::Tn10	Tc ^r Km ^r	H. Meade
pJB28	ColE1	pJC307 + Km ^r from pML21	Km ^r	This paper
pJB56	RSF1030	RSF1030 + Km ^r from pML21	Ap ^r Km ^r	This paper
pJB55	RSF1030 + ColE1	RSF1030-pML21	Ap ^r Km ^r	This paper

Primary double transformant cells replicate both plasmids

The ability of plasmids, temperature-sensitive for replication, to give rise to transformant colonies at the non-permissive temperature for plasmid replication was tested: pJB28, a ColE1 derivative containing the same genes for Km^r as pML21, and pHM15, a ColE1 derivative containing the Tc^r gene (see Table 1), were used to transform the strain HB101. The transformed cells were plated directly at 32 °C and 42 °C over a range of drug concentrations. At drug concentrations adequate to select Tc^r or Km^r, no transformants were obtained at the non-permissive temperature (42 °C), in spite of the fact that the plasmids were entering the cells (as witnessed by the growth of many transformants at 32 °C). Thus, a single copy of a plasmid was not sufficient to allow the growth of a visible colony.

Figure 3 shows that replication of both plasmids must occur in the primary transformed cell. When pJB28 transformants were preincubated at 32 °C before plating at 42 °C, the number of Km^r colonies which formed depended on the length of the preincubation time at 32 °C. The maximum number of colonies was obtained after a preincubation period of 180 min. On retesting, however, less than 0.1% of the cells in colonies forming at 42 °C were Km^r. This experiment showed that a plasmid-containing cell will form a colony on media that selects for a plasmid-coded drug resistance only if plasmid replication is allowed to occur in the cell before plating. Another observation, supporting the view that double transformant colonies arise as a consequence of a period of replication of both plasmids in the primary double transformant, is that the double transformant colony size is inversely related to the length of time that the cell population is grown unselectively before selective plating. This indicates that the number of viable daughters which the plated cell produces depends on how close in the cell lineage it is to the primary transformant.

A site model for plasmid incompatibility

The results described above have led us to the conclusion that when pMB9 and pML21 are used to simultaneously transform *E. coli*, both plasmids replicate in the primary transformed cell.

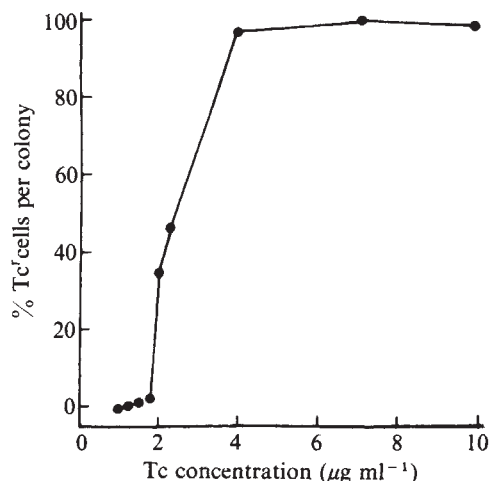


Fig. 2 Plasmid composition of daughter cells of double transformants. pMB9-pML21 plasmid double transformants of HB101 were prepared as described in Fig. 1 legend. Doubly transformed cells were selected on LB agar plates containing Km (20 μg ml⁻¹) and Tc (1–10 μg ml⁻¹). Colonies appearing on double selection plates were streaked onto LB agar plates and grown at 37 °C for 20 h. For each datum point, a minimum of 100 colonies were tested for resistance to Tc and Km.

In subsequent daughter cells only pMB9 or pML21 is able to replicate. The replicon hypothesis of Jacob *et al.*¹ can explain these results. Specifically, we propose that *E. coli* contains a site or sites at which ColE1 plasmids are replicated and that this is the basis of multicopy plasmid incompatibility. Once in occupancy, the resident plasmid at a site is not readily displaced by another ColE1 plasmid in the cytoplasm. Before cell division, the replication site or sites are duplicated and occupied by plasmid molecules that are the direct descendants of the plasmids occupying the parent sites. Any two ColE1 derivatives are incompatible because only a single plasmid molecule has access to each replication site.

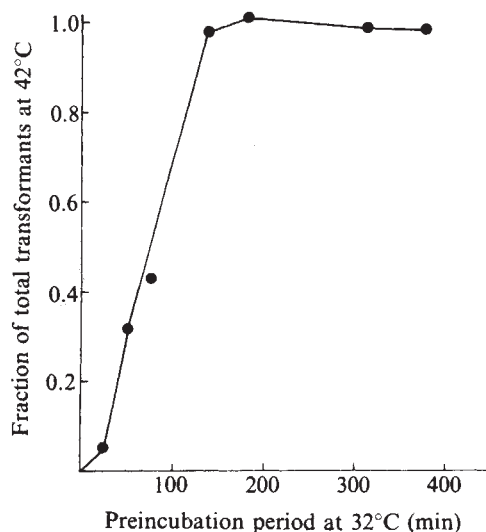


Fig. 3 Growth of pJB28 transformants at 32 °C and 42 °C. pJB28 was constructed by ligation, *in vitro*, of *Eco*RI linearised pJC307 with the *Eco*RI fragment of pML21 containing the genes for Km^r. Purified pJB28 plasmid DNA at a concentration of 5 μg ml⁻¹ was used to transform HB101 as described in Fig. 1 legend except that the heat pulse was at 32 °C and the cells were not grown in broth before plating. Aliquots of the transformation mixture were grown on LB agar plates containing 10 μg ml⁻¹ Km at 32 °C and, at the indicated times, transferred to 42 °C. Colonies were counted after 24 h of growth at 42 °C. As a control, several plates were incubated at 32 °C only.

In its simplest form, this site model predicts that *E. coli* contains a single site for ColE1 plasmid propagation. The results presented above indicate that both pMB9 and pML21 replicate in primary transformants. To reconcile this result with a single site replication model, it is necessary to propose that some fraction (about 1–5% based on Fig. 1) of a logarithmically growing population of *E. coli* cells transiently contain two sites for ColE1 propagation. Such cells may have duplicated the single site but not undergone cell division at the time of transformation by plasmid DNA. Double transformants could arise if

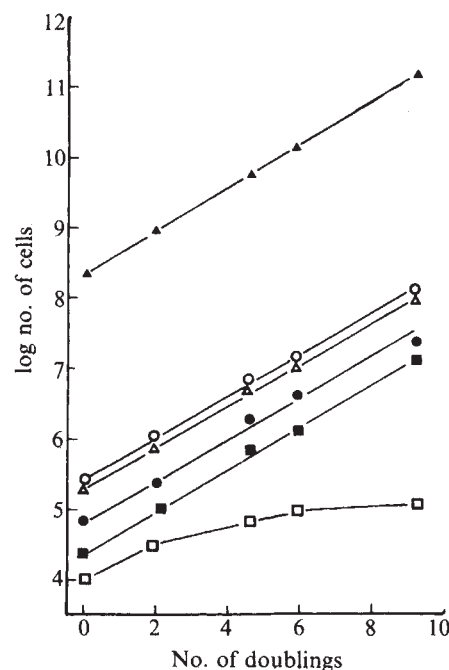


Fig. 4 Kinetics of segregation of pMB9 and pML21 from pMB9-pML21 double transformants. pJB56 was constructed by joining a derivative of RSF1030 containing an *Eco*RI site with the *Eco*RI fragment of pML21 containing the gene for Km^r. Mixtures of pMB9 and pJB56 DNAs or mixtures of pMB9 and pML21 DNAs (all at 20 μg ml⁻¹) were incubated with 10⁸ CaCl₂-treated HB101 cells. After incubation on ice for 60 min, the cells were heated to 42 °C for 2 min, diluted 10-fold in LB, and incubated at 37 °C for 20 min. Aliquots of transformed cells were plated on broth plates and on plates containing 10 μg ml⁻¹ Tc, 20 μg ml⁻¹ Km, and 10 μg ml⁻¹ Tc + 20 μg ml⁻¹ Km. The remaining cells were diluted fivefold in LB and grown for two doublings at which time the plating was repeated. This last procedure was repeated several times to give about 10 generations of growth. Plates were incubated at 37 °C for 30 h and all visible colonies were counted. Total cells (▲), pMB9 (○), pML21 (△), pJB56 (●), pMB9+pJB56 (■), pMB9+pML21 (□).

pMB9 occupies one and pML21 occupies the other site of such cells. Both plasmids could then replicate within this single cell until the point at which cell division occurs and the two sites are segregated. After cell division pMB9 continues to be replicated and propagated in one of the daughter cells and its progeny, and pML21 in the other daughter cell and its progeny. The random segregation of the non-site bound copies of both plasmids and their protein products enables the transitory maintenance of the double transformant phenotype.

Alternatively, *E. coli* may contain more than one replication site for ColE1 plasmids and double transformants could arise simply by two different plasmids acquiring one of the multiple sites. For a multiple site model to be in keeping with our data it is necessary to assume that the sites are segregated in a linear directive fashion. On cell division each site is duplicated, maintaining the linear arrangement with respect to one another. Half the linear array of sites are segregated to one daughter cell and half are segregated to the other daughter cell.

We present additional results below which support a replication site model as the basis of ColE1 incompatibility.

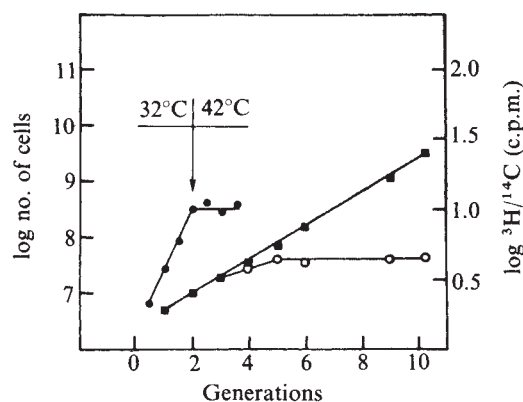


Fig. 5 Kinetics of segregation of pJB28 plasmid. *E. coli* strain FMA119 ($F^{-}su^{-}gal^{-}Sm^{R}recA^{-}thyA^{-}deo^{-}$) containing the temperature-sensitive replication plasmid pJB28 was grown in LB at 32°C or 43°C through ~10 doublings of the total population. Logarithmic growth was maintained throughout by successive dilution of the culture. At times indicated, aliquots of the culture were removed and plated on LB agar containing Km ($20 \mu\text{g ml}^{-1}$). In a separate experiment, FMA119 cells containing pJB28 were grown in 50 ml of minimal media supplemented with $2 \mu\text{g ml}^{-1}$ thymine and $20 \mu\text{g ml}^{-1}$ ^3H -thymine. Cells were grown for 2 doublings at 32°C in the presence of the label then diluted by a factor of 5 in the same media and grown at 43°C for 2 doublings. At the times indicated 10-ml samples were removed and mixed with equal aliquots of FMA119 cells grown at 32°C containing pJB28 which had been labelled with ^{14}C -thymine. The cells were pelleted, washed in 10 ml cold 10 mM Tris-HCl, pH 8.0, 1 mM EDTA and resuspended in 0.2 ml 20% sucrose containing 10 mM Tris-HCl pH 8.0. The cells were incubated with lysozyme (2 mg ml^{-1}) for 5 min at 4°C, 0.25 ml 0.25 M EDTA was added, the mixture was incubated at 4°C with occasional gentle mixing, 0.25 ml of lysis buffer containing 1% Brij 58, 0.4% sodium deoxycholate, 6.25 mM EDTA and 10 mM Tris-HCl pH 8.0 was added, and the mixture was incubated at 45°C for 2.5 min. The lysed cells were centrifuged at 17,500 r.p.m. for 60 min at 4°C in a Sorvall SS34 rotor in an RC2B centrifuge. The supernatant solution was extracted with equal volumes of phenol and chloroform. After an additional chloroform extraction, the aqueous phase was precipitated with ethanol. The precipitate was washed with 65% ethanol, dried, resuspended in 0.2 ml H_2O and mixed with 1 μg purified pJB28 DNA. The samples were applied to 1% agarose tube gels ($1 \text{ cm} \times 12 \text{ cm}$) and electrophoresed at 3 mA per gel for 14 h. Electrophoresis conditions, gel staining and photography were as described by Beedbrook and Bogorad¹⁷. The fluorescence corresponding to supercoiled pJB28 DNA was sliced from the gel, soaked in aquafuor for 16 h, and assayed in a liquid scintillation counter. The radioactivity incorporated into pJB28 DNA at 32°C and 43°C are superimposed with the results obtained for pJB28 segregation at 32°C and 43°C. ●, Ratio of ^3H c.p.m. in pJB28 DNA to the standard amount of ^{14}C c.p.m. in pJB28 DNA. ■, Number of cells plating on Km plates after growth at 32°C. ○, Number of cells plating on Km plates after growth at 42°C.

The kinetics of segregation of double transformants

Figure 4 illustrates experiments in which the kinetics of the segregation of pMB9-pML21 double transformants were compared with those of pMB9-pJB56 (a Km^r -conferring derivative of RSF1030) double transformants. Double transformants were constructed as described in Fig. 1 legend. The total cell population used in the transformation was then grown logarithmically without selection for about 10 doublings. At various times, samples were plated to assay total cell number and the presence of the two plasmids. The fraction of the total population transformed by pMB9 or pML21 was about 10^{-3} and the fraction transformed by pJB56 was about 2×10^{-4} . The number of pMB9-pML21 double transformants was about 10% that of the single transformants. This number is likely to be an underestimate of the absolute number of primary double transformants as some segregation must occur during the unselective growth period before the first plating. The total cell population grew logarithmically throughout the experiment and no loss by segregation of single pMB9 or pJB56 transformants or double pMB9+pJB56 transformants was observed after 10 generations. pMB9-pML21 double transformants increased initially, in a non-log-linear fashion, to give ~10 times the

number of "doubly transformed" cells. After six doublings of the total cell population, the number of pMB9-pML21 double transformants had plateaued. These data corroborate the experiments described above which showed that pMB9-pML21 double transformants are unstable and can only exist for short periods of time.

Figure 5 shows that the kinetics of segregation of pMB9-pML21 double transformants are similar to the kinetics of distribution of pJB28 to daughter cells following a shift to the nonpermissive temperature for pJB28 replication. At 32°C, both the growth of Km^r cells and the replication of pJB28 (as shown by ^3H -thymidine incorporation in plasmid DNA) are log-linear. After transfer to 42°C, plasmid DNA synthesis stopped within the temperature shift and the next measurement of plasmid synthesis (20 min). In contrast, the number of Km^r -resistant cells increased in a non-linear fashion during the first four doublings of the total cell population at 42°C, at which point the number of Km^r cells plateaued.

Comparison of Figs 4 and 5 shows that the extent of growth of pJB28-containing cells following a shift to 42°C is similar to the increase in pMB9-pML21 double transformants immediately following transformation. This relationship suggests that the 10-fold increase in pMB9-pML21 double transformants was due to the distribution to daughter cells of pML21 and pMB9 plasmids which were produced in the primary transformed cell. These kinetic observations are consistent with a site model of plasmid replication because they indicate extremely rapid cessation of replication of one of the two plasmids in the daughter cells of the primary transformant. This leads rapidly to a steady state condition in which there is no net increase in double transformants. Moreover, the 'double transformants' obtained are genetically unstable and cannot be maintained even by selection. These observations are not consistent with a

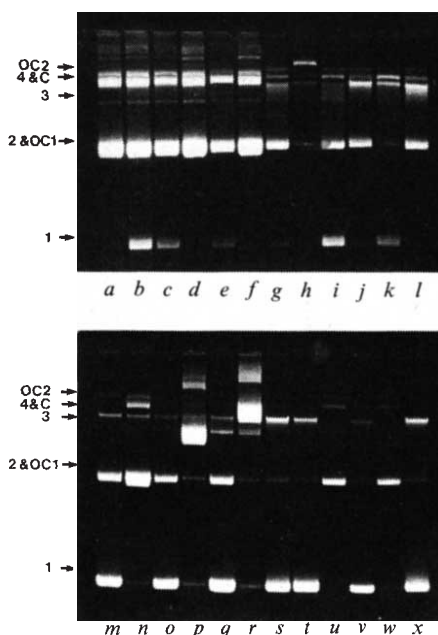


Fig. 6 *E. coli* propagates only one multimer form of pMB9 per cell. *E. coli* strains JC1157 and JC1157 *recA* ts (ref. 16) were transformed with pMB9 monomer DNA as described in Fig. 4 legend. Transformed cells were plated at 32°C on LB agar containing $10 \mu\text{g ml}^{-1}$ Tc. Cells from colonies of JC1157 (pMB9) and JC1157 *recA* ts(pMB9) grown at 32°C were plated again at 32°C and 42°C. Six colonies from each of the four platings were used to prepare plasmid DNA as described in Fig. 5 legend. Plasmid DNA samples were electrophoresed in 1% agarose gels. Electrophoresis is from top to bottom. pMB9 DNA from various colonies of JC1157 grown at 32°C (lanes a-f), from JC1157 grown at 42°C (lanes g-l), from JC1157 *recA* ts grown at 32°C (lanes m-r), and JC1157 *recA* ts grown at 42°C (lanes s-x). pMB9 supercoiled monomers (1), pMB9 supercoiled dimers (2), pMB9 supercoiled trimers (3), pMB9 supercoiled tetramers (4), pMB9 open circular monomers (OC1), pMB9 open circular dimers (OC2), and chromosomal DNA (C).

model which invokes copy number repressors as the basis for plasmid incompatibility because repressor models predict an indefinite log-linear increase in the number of double transformants. With a positive selection for both plasmids one expects to maintain an increasing lineage of cells containing both plasmids⁶, even in an extreme case in which two incompatible plasmids have very different affinities for the replication repressor.

Transformation of cells containing resident plasmids

Table 2 lists the transformation frequencies obtained when pMB9 was used to transform HB101 containing various resident plasmids. In this experiment the transformation frequency of HB101 by pMB9 was 10^{-5} per cell. This frequency was reduced by more than three orders of magnitude when HB101 contained pML21 but was unaffected by the residency of pJB56, a compatible plasmid. Residency by pJB55, a hybrid replicon between RSF1030 and pMB9, reduced the transformation frequency by pMB9 to a similar extent as that caused by pML21, but residency by pMB9 did not affect the transformation frequency of pJB55.

These data can be explained by a site model for incompatibility. As discussed above, the exchange of plasmids at the replication site is postulated to be an infrequent event. Thus, the accessibility of the site to an incoming plasmid would be greatly reduced if the site were already occupied by an incompatible resident plasmid. The hybrid replicon pJB55 could reside at both the sites for ColE1 and RSF1030 replication. When in residence, pJB55 would be expected to prevent pMB9 from acquiring the site. On the other hand, the hybrid plasmid should be able to acquire the RSF1030 site when pMB9 is the resident plasmid.

We have found that pMB9-pJB55 double transformants are propagated stably and indefinitely. This result is not consistent with a replication repressor model for plasmid incompatibility as repressor synthesised by the ColE1 portion of the RSF1030-ColE1 hybrid replicon would be expected to induce the segregation of pMB9.

Physical evidence for a directive segregation model

Experiments shown in Fig. 6 provide physical evidence that *recA*⁻ *E. coli* is capable of replicating only a single ColE1 plasmid per cell. for this experiment we took advantage of the following: (1) pMB9 plasmid molecules undergo reciprocal recombination in *recA*⁺ cells to form plasmid multimers at high frequency¹⁴. In contrast, multimer forms are stably propagated in *recA*⁻ *E. coli*. (2) A temperature sensitive (ts) *recA*⁻ mutant of *E. coli* forms plasmid multimers when the plasmid-containing strain is grown at 32 °C but not at 42 °C.

In the experiment shown in Fig. 6, the fate of plasmid multimers formed and present in the cells of the ts *recA*⁻ strain grown at 32 °C, was followed when the temperature was shifted to 42 °C. As a control, *recA*⁺ strain, otherwise isogenic with the ts *recA*⁻ strain was used. Cultures growing at 32 °C were plated for single colonies at 32 °C and 42 °C and plasmid DNAs derived from single colonies were examined by gel electrophoresis. For the *recA*⁺ strain (Fig. 6a-l), six colonies analysed at 32 °C and 42 °C all contained different multimer forms. Various multimers were also seen in DNA preparations from the ts *recA*⁻ colonies plated at 32 °C (Fig. 6m-r). When the ts *recA*⁻ strain was plated at 42 °C, however, individual colonies contained only one plasmid size class (Fig. 6s-x): four contained monomers and two contained dimers. That is, the ts *recA*⁻ strain propagated only one of the possible size classes of pMB9 plasmid molecules at 42 °C despite the fact that the cells growing at 32 °C contained various multimer forms.

Table 2 Effect of resident plasmids on transformation frequency

Transforming plasmid	Resident plasmid	Transformation frequency per cell
pMB9	None	10^{-5}
pMB9	pML21	$<10^{-8}$
pMB9	pJB56	10^{-5}
pMB9	pJB55	$<10^{-8}$
pML21-RSF1030 hybrid (pJB55)	pMB9	10^{-6}

Strain HB101 containing the indicated plasmids was grown in LB media containing $20 \mu\text{g ml}^{-1}$ Km or $10 \mu\text{g ml}^{-1}$ Tc as appropriate. Approximately 10^9 cells were transformed with $1 \mu\text{g}$ of the indicated plasmid DNAs as described in Fig. 1 legend. Transformants were grown in LB media containing $10 \mu\text{g ml}^{-1}$ Tc or $10 \mu\text{g ml}^{-1}$ Km to select the resident plasmid and then plated on LB solid medium containing $10 \mu\text{g ml}^{-1}$ Tc and/or $20 \mu\text{g ml}^{-1}$ Km to select both the resident and the incoming plasmid. The transformation frequency indicated is the number of cells containing both resident and incoming plasmids divided by the total number of cells used in the transformation.

Discussion

We have presented evidence that strongly supports the view that incompatibility between ColE1 plasmids is a direct consequence of competition between plasmids for a single or a limited number of plasmid replication site(s). Our results contrast other studies^{6,8} which show that *E. coli* strains are capable of maintaining two ColE1 plasmid replicons over many generations. The results of these studies are best explained if plasmid incompatibility is envisaged to be the consequence of transacting repressors which normally regulate plasmid copy number. A significant difference in the experimental design of these previous studies and the experiments described here is that we have used *recA*⁻ strains. We have shown previously that reciprocal recombination occurs between plasmid molecules in *recA*⁺ (but not in *recA*⁻) *E. coli* leading to the formation of plasmid multimers¹⁴. Recombination occurs only at regions of homology and is reversible. We showed that stable double transformants can be obtained when pMB9 and pML21 are used to simultaneously transform a *recA*⁺ strain. These doubly transformed strains contain hybrid pMB9-pML21 plasmids¹⁴.

The phenomenon of plasmid-plasmid recombination can be invoked to reconcile our observations with previous studies. Assume that a *recA*⁺ strain is replicating a pMB9-pML21 hybrid plasmid (formed *in vivo* by recombination) at a ColE1 replication site. Occasionally, intramolecular recombination of the site bound hybrid plasmid will occur and will result in occupation of the site by pMB9 or pML21 instead of the pML21-pMB9 hybrid. From this point on, the site will only replicate pMB9 or pML21 and one of the two plasmids may be segregated away unless it regains access to the replication site by recombination.

Warren and Sherratt⁶ found that smaller ColE1 plasmids with high copy numbers usually displaced larger, low copy number plasmids. This observation can also be explained by plasmid-plasmid recombination. Assume that a hybrid ColE1 replicon is comprised of two plasmids, which, as monomers, are present in different copy numbers. Following intramolecular recombination and cell division, daughter cells carrying the 'high copy' replicon at the replication site will contain a high ratio of 'high copy' monomers to hybrids. In comparison, daughter cells which contain the 'low copy' replicon at the replication site, will contain a lower ratio of monomers to hybrids. The higher the ratio of monomers to hybrids, the lower the probability that the replicating monomer at the site will recombine with a hybrid plasmid to restore the double transformant genotype. A similar argument can be made for large versus small plasmids. A small site bound plasmid would have less extensive homology with the hybrid plasmids in the cytoplasm than would a large site-bound plasmid. This decreases the probability that a small site-bound plasmid would recombine with a cytoplasmic hybrid plasmid, increasing the probability that the small plasmid would displace the larger one. That is, recombination may be the basis of incompatibility hierarchies among ColE1 replicons⁶; if so such hierarchies would not be expected to be observed in *recA*⁻ strains.

We conclude that plasmid incompatibility is the consequence of ordered, directive segregation of replication sites. For pMB9 and pML21 plasmids attachment to the replication site seems to be irreversible. Certain ColE1 plasmid derivatives appear to segregate from *E. coli* strains spontaneously (Ausubel and Ow, unpublished). Presumably such plasmids have an altered affinity for the replication site, compared with say pMB9, such that the binding with the site is unstable. For these plasmids the affinity for the replication site and copy number control would be important in controlling incompatibility. Take for example a primary double transformant *recA* cell containing a 'stable' attachment plasmid at one site and an 'unstable' attachment plasmid at another site; daughter cells sufficiently distant in the cell lineage from the primary cell to have completely segregated

the replication sites may still contain non-site-bound plasmids of both types. If at this point the 'unstable' attachment plasmid was uncoupled from a replication site exchange with the stable attachment plasmid may occur thus switching the plasmid 'genotype' of the cell. Hierarchies of incompatibility could be generated by variable affinities of various plasmids for the replication sites.

We thank D. Helinski for providing pJC307 before its publication and a referee for drawing our attention to the potential importance of attachment bias of plasmids to replication sites. This work was supported by NSF grant PCM75-21435 to FMA. J.R.B. was a Maria Moors Cabot Research Fellow and an EMBO Long Term Fellowship holder during the course of this work. H.L. was supported by NIH grant HD-01229 to Dr P. Doty.

Received 5 April; accepted 9 August 1979.

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Three new types of viral oncogene of cellular origin specific for haematopoietic cell transformation

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The RNAs of seven replication-defective leukaemia virus (DLV) strains contain three types of unique sequences, which correlate with the capacity of a given virus strain to transform erythroblasts, macrophage-like cells and myeloblasts, respectively. These sequences, termed erb, mac and myb, have their counterparts in the normal DNA of avian and mammalian species. Our results indicate that DLVs represent recombinants between a common 'vector' related to a chicken endogenous virus and one of three types of cellular gene possibly involved in haematopoietic differentiation.

ON the basis of their oncogenic properties, avian retroviruses are assigned to either of two major classes. Of many characterised natural field isolates, most (non-defective avian leukaemia viruses, or ALVs) are 'weakly oncogenic' and cause predominantly lymphatic leukaemia after a latency period of months to years following inoculation into susceptible strains of chickens. The ALVs most frequently occur as independent agents containing all the genetic information required for viral replication. In contrast, several avian retroviruses are highly oncogenic, causing disease after a latency period of days to weeks. These include the replication-competent avian sarcoma viruses (ASVs, or Rous sarcoma viruses) which readily induce sarcomas in susceptible birds and transform fibroblasts *in vitro*,

and also the replication-defective leukaemia viruses (DLVs) which require helper ALVs for propagation in cultured cells (for review, see ref. 1).

The seven available independent isolates of DLVs have been assigned to three subgroups based on the types of neoplasm they induce and on the differentiation phenotypes of haematopoietic cells they transform *in vitro*^{1,2}. (1) The avian erythroblastosis-type viruses (AEV strains R and ES4 are probably identical³) induce erythroblastosis *in vivo* and transform erythroblasts *in vitro*. (2) The avian myelocytomatosis-type viruses (MC29, CM1, OK10 and MH2) transform macrophage-like cells *in vitro* and some strains induce myelocytomatosis *in vivo*. (3) The avian myeloblastosis-type viruses (AMV and E26) induce myeloblastosis *in vivo* and transform myeloblasts *in vitro*. AEV can also induce sarcomas and transforms fibroblasts *in vitro*, and MC29-type viruses can induce carcinomas and occasional sarcomas and transform epithelial cells and fibroblasts *in vitro*^{1,2}. In this report we have investigated whether the oncogenicity of these viruses could be related to the presence of specific nucleotide sequences in their genome. Such a relationship would imply that part or all of these specific sequences represent new viral oncogenes.

DLVs are related to non-defective avian leukaemia viruses

DLVs were originally isolated from chickens in association with their natural replication-competent helper viruses belonging to the ALV group. Attempts to complement DLVs with helpers unrelated to ALVs have failed⁴. A possible homology between certain genomic sequences of the DLVs and ALV genes⁵⁻⁷ was

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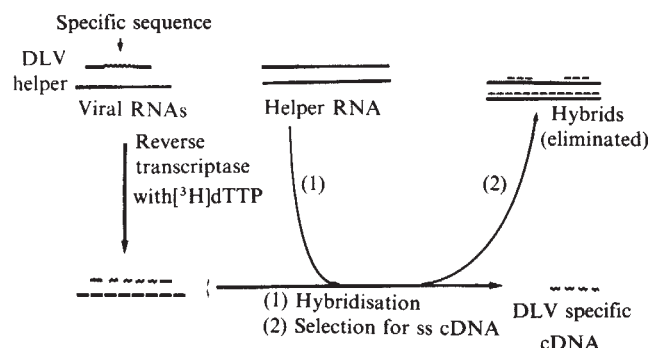


Fig. 1 Scheme for the preparation of DLV-specific cDNAs. Cloned AEV (RAV-2) and MC29 (RAV-2) pseudotypes, obtained by rescuing these viruses with RAV-2 from cloned non-producer fibroblasts, were propagated in chicken fibroblasts. Viral RNAs were extracted from the pelleted viruses, then transcribed into labelled cDNA using *in vitro* reverse transcription with purified AMV reverse transcriptase, ^3H -dTTP and unlabelled dCTP, dGTP, dATP and calf thymus priming. In a negative selection step each cDNA mixture was hybridised to an excess of RNA extracted from RAV-2 grown separately. Single-stranded DLV-specific cDNAs were then isolated after fractionation on hydroxyapatite. AMV-specific cDNA was prepared in a similar way, but using RNA of AMV (helper) pseudotype obtained from the plasma of infected chickens, and a mixture of ALV helper RNAs extracted from RAV-1, RAV-2 and MAV-2 (0) viruses for the selection. Non-hybridisable radioactivity was eliminated from the cDNAs by a positive selection with homologous RNAs⁸ (not shown). Specific activities of the ^3H -cDNAs were $\sim 50 \times 10^6$ c.p.m. per μg . Details of these experiments will be described elsewhere.

Table 1 DLV genomes in nonproducer cells are related to ALVs

DLV strain	Type of nonproducer cell	Annealing (% S_1 resistance) with	
		cDNArep	cDNAsarc
AEV	chicken fibroblast	28	<3
MC29	quail fibroblast	45	<3
CMII	quail fibroblast	41	<3
OK10	quail fibroblast	77	<3
MH2	quail fibroblast	32	<3
AMV	chicken myeloblast	50	<3
E26	chicken myeloblast	36	<3

Total RNA was extracted from the different avian nonproducer cells and hybridised in stringent conditions (25- μl aliquot containing 1–10 mg ml^{-1} RNA, 0.6 M NaCl, 68 °C) with cDNAs (1,000 c.p.m. per point = 0.02 ng) to plateau C_{0t} values (defined as $10 \times$ the $C_{0t1/2}$). cDNArep and cDNAsarc were prepared as described earlier³, but using calf thymus-primed polymerase reactions¹⁴. Hybrids were assayed by S_1 nuclease. No correction for the expression of endogenous RAV-O or cellular sarc was made as they gave values below 3% in each case.

values to several viral RNAs. The results (Table 2) allow several conclusions to be drawn. First, the cDNAs tested are specific for the DLVs used for their preparation. Second, they represent distinct sets of sequences, unrelated to each other by cross-hybridisation experiments, and third, cDNA_{mc29} shows extensive homology to RNA of CMII, OK10 and MH2, as does cDNA_{amv} to E26 RNA. As will be described elsewhere, the three cDNAs exhibited exactly the same specificity when tested with cellular RNAs extracted from nonproducer cells, further demonstrating that the probes indeed detect sequences specific for the three types of DLV.

To investigate the origin of the DLV-specific sequences, DNA extracted from normal avian cells was tested for its ability

investigated by testing clones of avian cells containing the DLV provirus without its helper. Such cells, usually unable to produce virus⁶, still contain several RNA transcripts of the DLV provirus. We tested whole RNA of such nonproducer cells, using molecular hybridisation with labelled probes representing a homogeneous transcript of an ALV, td Pr-B (cDNA_{rep}), or of the *src* gene of ASVs (cDNA_{sarc})⁸. Table 1 indicates that all the DLV genomes tested contain ALV-related nucleotide sequences in different amounts (28–77%), but lack the *src* gene of ASV^{9,10}. However, the detected homology to ALV cannot account for the entire genetic content of the DLVs, which all contain 6,000–8,000 nucleotides in their 28–34S genome^{11,12}. Thus, these viruses may contain sets of nucleotide sequences unrelated to ALVs.

DLV-specific sequences

We looked for specific sequences by analysing the prototype strain of each of the three DLV subgroups, namely AEV, MC29 and AMV. Clone viral strains were obtained for AEV and MC29 by superinfection of nonproducer cells with the plaque-purified helper virus RAV-2. The rescued viruses were then tested for their oncogenic potential *in vitro* (using the bone marrow transformation assay²) and propagated in chicken erythroblasts (for AEV) or chicken fibroblasts (for MC29). AMV was obtained in high yields from the plasma of infected chickens.

The preparation of specific cDNAs of these viruses (Fig. 1) was similar to the selection of cDNA specific for the *src* gene of ASVs⁸. cDNAs of AEV, MC29 and AMV were selected so that they could still hybridise to their RNAs of origin, but not to the RNA of the helper viruses used to grow the viral stocks or any of the other ALVs tested. These specific cDNAs were denoted cDNA_{aev} for AEV, cDNA_{mc29} for MC29 (ref. 13) and cDNA_{amv} for AMV.

The distribution of viral nucleotide sequences related to these specific cDNAs was explored by hybridising them at plateau C_{0t}

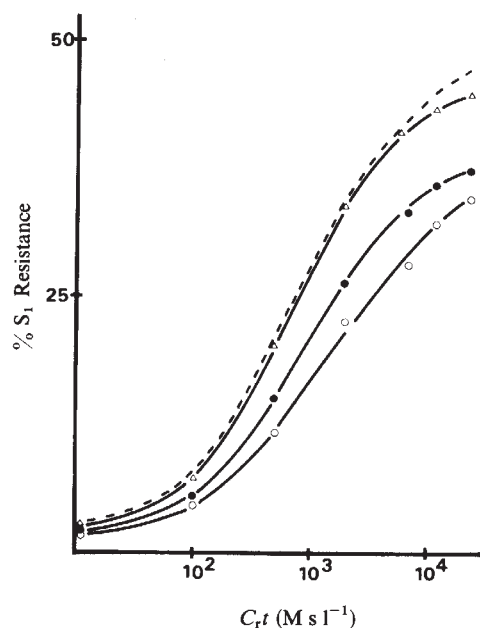


Fig. 2 DLV-specific sequences have their counterparts in normal chicken DNA. Aliquots of 11-day chicken embryo DNA (200 μg per point, sheared to 4–6S) were hybridised with the different specific cDNAs (1,000 c.p.m. per point = 0.02 ng) in stringent conditions (0.6 M NaCl, 68 °C) at increasing C_{0t} values. Hybrids formed were tested for their resistance to S_1 endonuclease. ●—●, cDNA_{aev}; △—△, cDNA_{mc29}; ○—○, cDNA_{amv}; ----, cDNAsarc. Similar results were obtained with normal quail embryo DNA (not shown).

Table 2 DLVs contain unique nucleotide sequences

RNA derived from	Annealing (% S ₁ resistance) with		
	cDNA _{aev}	cDNA _{mc29}	cDNA _{amv}
AEV	100	<3	<3
MC29	<3	100	<3
CMII	<3	96	<3
OK10	<3	91	<3
MH2	<3	66	<3
AMV	<3	<3	100
E26	<3	<3	68
ALV helpers (control)	<3	<3	<3

Viral 50–70S RNAs were extracted and hybridised to plateau C_{ot} values ($>10 \text{ M s l}^{-1}$) in stringent conditions (0.6 M NaCl, 68 °C) with the specific cDNAs (1,000 c.p.m. per point = 0.02 ng). The hybrids formed were tested for S₁ nuclease resistance. Results obtained were standardised to the values of the homologous reaction. Maximum hybridisation of cDNA_{aev} with AEV RNA was 95%; that of cDNA_{mc29} with MC29 RNA 90% and that of cDNA_{amv} with AMV RNA 95%. The DLVs tested were cloned pseudotypes with RAV-1 or RAV-2 helper ALV, with the exception of AMV and E26, which were not cloned. The ALV helper RNAs tested as controls were obtained from RAV-1, RAV-2, RAV-49, RAV-50, MAV-2 (0). The numbers boxed indicate the important homologies.

to hybridise with cDNA_{aev}, cDNA_{mc29} and cDNA_{amv}. Figure 2 shows that all three types of sequences have their counterparts in normal cellular chicken DNA and, as judged from the $C_{ot_{1/2}}$ values, that they are present in the non-repetitive DNA at one or two copies each per haploid genome. The low plateau values observed in Fig. 2 are due to the competition of DNA reassociation with the DNA–cDNA hybridisation reaction; transcription analyses (see below) show that most or all of the specific sequences are present in normal chicken DNA. Results obtained with molecular hybridisation in liquid phase in stringent conditions show that the bulk of the non-repetitive chicken DNA sequences have been lost relatively rapidly during evolution: labelled chicken unique sequence DNA barely anneals (Fig. 3) to the DNA of species from another genus. For example, quail and chicken, which probably evolved from a common ancestor some 20–40 Myr ago, have DNAs that share less than 25% homology when cross-hybridised (Fig. 3). Similarly, ALV-related ‘virogene’ sequences have recently been shown to be absent in some species of the Galliformes¹⁴. In contrast, cellular sequences related to cDNA_{aev}, cDNA_{mc29} and cDNA_{amv} have been highly conserved throughout the phylogeny of the higher vertebrates. As also shown in Fig. 3, their ‘evolutionary half lives’ are comparable to those of known stable genes such as globin or ovalbumin. The latter values resemble those obtained earlier for the transforming gene *src* of ASVs^{15–17} and agree with the results recently described for MC29 by Sheiness *et al.*¹⁸.

As normal cells contained nucleotide sequences related to highly transforming viruses, we studied the rate of transcription of such sequences. Total RNA extracted from normal chicken (or quail) fibroblasts was hybridised to the different specific cDNAs. Figure 4 shows that these sequences are all transcribed at a low level (a few copies per cell), as previously found for the expression of ‘cellular *src*’ (refs 16, 19). Cellular sequences related to cDNA_{aev} are transcribed at two or three copies per cell; cellular sequences related to cDNA_{mc29} are reproducibly expressed at a higher level (5–10 copies per cell), as Sheiness *et al.*¹⁸ found. In contrast, cellular sequences related to cDNA_{amv} are transcribed at lower levels in fibroblasts (one copy per cell). As can be seen in Fig. 4, all these cellular sequences reach plateau hybridisation values close to 100%, and must therefore be essentially fully represented in normal cellular nucleic acids.

Three new oncogenes

Our results show that all seven defective leukaemia viruses studied contain part or all of one out of three different sets of specific nucleotide sequences complementary to cDNA_{aev}, cDNA_{mc29} or cDNA_{amv}, which have their counterpart in normal cells of higher vertebrates. None of these sequences is related to the transforming gene *src* of ASVs. Although it is not understood how recombinants between ALV sequences and cellularly derived sequences can acquire oncogenic capacity, several indications favour the hypothesis that three specific transforming genes, termed *erb*, *mac* and *myb*, are detected by cDNA_{aev}, cDNA_{mc29} and cDNA_{amv}, respectively. (1) There is a strict correlation between the presence of *erb*, *mac* or *myb* sequences in a given virus and its selective capacity to generate transformed erythroblasts, macrophage-like cells or myeloblasts^{1,2,20}. (2) The properties of these sequences and their origin are reminiscent of results obtained earlier for the transforming gene *src* of ASVs¹⁵: size, cellular origin and phylogenetic stability^{16,17}. (3) As indicated by heteroduplex mapping studies with MC29 (ref. 21), these sequences do not appear as bits of sequences randomly distributed throughout the viral RNA, but rather as continuous stretches located inside the viral genome. (4) Temperature-sensitive mutants have recently been isolated from AEV, indicating that a virus-coded protein must be synthesised to maintain the undifferentiated state of transformed erythroblasts²². (5) All DLVs tested contain the 5' region of the ALV genome (partial *gag* gene¹²). In the strains studied so far (MC29 and MH2) this region was located adjacent to the DLV-specific sequence^{21,23}. In addition, cells non-productively transformed by AEV, MC29, CMII and MH2 express novel polypeptides of molecular weight 75,000 (ref. 3), 110,000 (refs 6, 7), 90,000 (ref. 7) and 100,000 (ref. 4), respec-

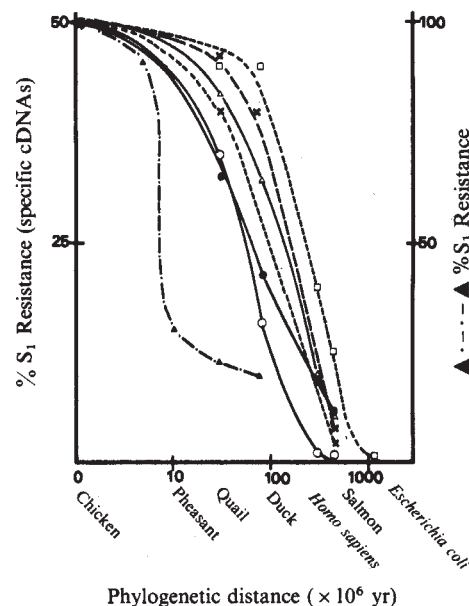


Fig. 3 Stability of cellular DLV-related specific sequences during evolution. Aliquots of DNA extracted from different species having diverged increasingly long ago with speciation were hybridised in the specific conditions described in Fig. 2. The values obtained were standardised arbitrarily, for comparison purposes, to 50% for the chicken, which was also taken as the origin of the scale indicating phylogenetic distance. ●—●, cDNA_{aev}; △—△, cDNA_{mc29}; ○—○, cDNA_{amv}; □—□, cDNAsarc. Also shown (▲—▲) are results obtained with ³H-labelled chicken unique sequence DNA¹⁴ (1,000 c.p.m. per point = 20 ng) in similar conditions (standardised to 100% for homologous reassociation) and with cDNAs representing genes known to be conserved throughout the evolution of vertebrates; ×—×, cDNA_{lgo} (ref. 16) for globin; ×—×, cDNA_{ova} (ref. 16) for ovalbumin.

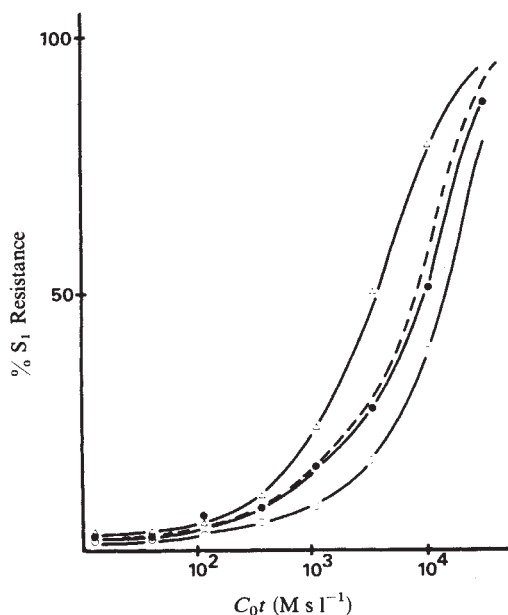


Fig. 4 Transcription of the cellular DLV-related specific sequences in normal fibroblasts. Total RNA was extracted from normal chicken embryo fibroblasts and hybridised at increasing C_{0t} values (varying RNA concentration up to 10 mg ml^{-1}) in stringent conditions (0.6 M NaCl , 68°C), to the specific cDNAs ($1,000 \text{ c.p.m. per point} = 0.02 \text{ ng}$). Hybrids were scored for their S_1 endonuclease resistance. Symbols are as in Fig. 2.

tively, with the structure: partial *gag-x* or *y*, where *x* or *y* represent polypeptide moieties which are antigenically unrelated to known ALV proteins, and presumably correspond to part or all of the specific *erb* or *mac* nucleotide sequences. That these polyproteins could be involved in the transformation process is indicated by the finding that the type of tryptic peptide pattern exhibited by the non-*gag* portion of the polyprotein of a given DLV closely correlates with the transformation specificity of the virus (M. J. Hayman, personal communication, and ref. 24). In their general structure and ability to code for *gag*-related polyproteins, avian DLVs resemble replication-defective mammalian retroviruses capable of inducing leukaemias or sarcomas with a short period of latency, such as the Abelson²⁵ murine virus and feline sarcoma virus^{26,27}.

Avian DLVs behave like recombinants between an ALV-related vector virus of low oncogenicity and unknown origin¹⁴ and an evolutionarily stable set of nucleotide sequences of cellular origin. In this regard they also closely resemble ASVs (for review, see ref. 11). The mechanism of such a recombination remains to be elucidated, but could be related to the jumping polymerase hypothesis proposed recently by Coffin²⁸. How frequently such a recombination occurs is not known, although about 30 DLV-like and 1–3 ASV-like independent isolates have

been reported². However, a transduction of cellular sequences would not necessarily lead to such severe effects as the generation of a highly oncogenic virus and may therefore be much more frequent than anticipated. Indeed, we have recently found in an ASV stock a new virus of unknown oncogenicity containing apparently unknown cellular genetic material (N. Pluquet and D.S., unpublished).

That only a partial homology was observed between the specific sequences of MC29 and MH2 as well as between the specific sequences of AMV and E26 (Table 2) can be explained in three ways—these viruses acquired cellular genes which already differed in their sequences; they acquired the same gene and the observed differences reflect a divergence introduced by passage of the virus; a second recombination event occurred in MH2 and E26. The finding that there are only one or two copies per haploid genome of these genes present in cellular DNA is consistent with the second possibility. The observation that there is a somewhat lower degree of homology of cDNA_{amv} to cellular DNA than that of the other types of specific cDNA tested (Fig. 2) might reflect the long passage history of AMV and would also agree with the second of the above interpretations. But hybridisation experiments between cDNA_{mc29} and MH2 RNA carried out in non-stringent conditions do not show the significant increase of annealing which the second interpretation would predict (M.R., S.S. and D.S., unpublished) and thus leave that question unanswered.

The role of DLVs in leukaemic transformation could be explained by a generalisation of the 'differentiation block' hypothesis^{22,24}: the oncogenes of DLVs are homologous to normal cellular genes ('cellular *erb*, *mac* or *myb*') coding for lineage-specific haematopoietic differentiation proteins; the transforming proteins act by competitively inhibiting the corresponding cellular proteins^{2,17}, thus leading to a block of differentiation and subsequent leukaemogenesis. This hypothesis would predict that RNA sequences corresponding to the cellular *erb*, *mac* and *myb* genes are preferentially expressed in normal erythroblasts, macrophages and myeloblasts, respectively. Spontaneous leukaemogenesis may then simply result from a malfunction of such genes. Indeed, several different genes may be able to induce the same type of leukaemia. In this respect, it would be interesting to know whether there are viruses causing the same type of leukaemia but containing different cell-related oncogenes. Such viruses would also be useful for elucidating why the DLVs also induce sarcomas or carcinomas, an additional specificity for non-haematopoietic tissues difficult to explain by the differentiation block theory.

We thank Drs B. Royer-Pokora and K. Bister for providing cells, N. Oker-Blom and L. Gazzolo for OK10 and MH2 viruses, M. Bellard and A. Therwarth for cDNAs of globin and ovalbumin, M. B. Raes for technical assistance, N. Devassine for typing, Drs C. Sherr, R. Marusyk and M. Hayman for helpful corrections and discussions. AMV polymerase, PR-C ASV and chicken plasma were provided through the NCI (Office of Resources and Logistics). This work was supported by INSERM (ASR-1-018 and CRL 77.5.054.2), CNRS (AI, ERA 225), DGRST (77.7.1879) and the Pasteur Institute of Lille.

Received 4 June; accepted 22 August 1979.

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T4 DNA topoisomerase: a new ATP-dependent enzyme essential for initiation of T4 bacteriophage DNA replication

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A novel ATP-dependent DNA topoisomerase which makes reversible double-strand breaks in the DNA double helix has been purified to near homogeneity from T4 bacteriophage-infected Escherichia coli cells. Genetic data suggest that this activity is essential for initiating T4 DNA replication forks in vivo.

THE process of DNA replication fork movement can be successfully duplicated *in vitro* and has been shown to require several different types of purified protein^{1,2}. In contrast, the process of replication initiation, whereby new replication forks are established at specific chromosome origins, has not yet been reconstituted with purified components. As a result, our knowledge of the enzymology of fork initiation is rather limited (for review see ref. 3).

Genetic studies of T4 bacteriophage development have indicated that the products of T4 genes 39, 52 and 60 probably interact with each other to form a complex, and that this complex is essential for normal T4 DNA replication⁴. When any one of these three T4 proteins is defective, replication still begins at about the same time as in the wild type, but the rate of the initial phase of DNA synthesis is reduced⁵. This slow rate of DNA synthesis results from a low incidence of replicative forks, rather than from any reduction of the fork movement rate⁶. The products of genes 39, 52 and 60 are thus believed to be involved in the initiation of T4 DNA replication⁶.

Here, we report the discovery, properties and purification of a new ATP-dependent DNA topoisomerase. DNA topoisomerase is the name suggested for a class of enzymes that catalyse the concerted breaking and rejoining of DNA backbone bonds, as these activities are invariably detected by their ability to promote the interconversion between different topological isomers of DNA (for review, see ref. 7). The T4-induced DNA topoisomerase characterised here seems to be composed of proteins coded for by T4 genes 39, 52 and 60. The properties of this enzyme suggest ways in which it can act to initiate replication forks at T4 chromosome origins. A similar complex of T4 proteins has been independently isolated by Stetler, King and Huang⁸, using an *in vitro* complementation assay to monitor gene 39 protein activity.

A new ATP-dependent DNA topoisomerase activity induced by T4 bacteriophage infection

Infection of *Escherichia coli* with T4 bacteriophage results in a marked increase in the ability of a cell lysate to relax covalently closed superhelical DNAs, a reaction which requires at least one transient cleavage of a DNA backbone bond⁹. This conversion

of DNA circles from a negatively supercoiled form to a less supercoiled form may be sensitively detected by agarose gel electrophoresis, as shown in Fig. 1 for circular duplex PM2 DNA which has been incubated with various dilutions of an extract. Supercoil-relaxing activity is detected in this crude lysate only after extensive dilution away from the endonucleases which are also present; these nucleases convert the initial DNA substrate (form I DNA) to nicked DNA circles (form II DNA) and unit-length linear DNA molecules (form III DNA). For this reason, a group of DNA bands characteristic of partially relaxed, covalently closed DNA circles (which have an electrophoretic mobility between that of form I and form II DNAs) could be detected only after a 540-fold extract dilution. Surprisingly, no DNA supercoil relaxation was detected when the rATP in the reaction mixture was omitted. This activity was not blocked by antibody to *E. coli* ω protein or affected by the *E. coli* DNA gyrase inhibitor novobiocin (data not shown). The possibility of DNA relaxation by an excess of DNA ligase in the presence of an endonuclease was ruled out, as the same level of supercoil-relaxing activity was found in extracts of cells infected with a T4 DNA ligase (gene 30) amber mutant (see Table 1). We therefore concluded that the activity detected in Fig. 1 originates from a previously unrecognised DNA topoisomerase. By analogy with other DNA topoisomerases, we assume that this enzyme catalyses both the breakage and the rejoining of DNA backbone bonds; moreover, it must do so in a concerted way, such that the putative intermediate in which a DNA backbone bond is broken exists only transiently. This requirement is necessitated by our failure to detect a population of nicked DNA circles as intermediates in reactions with the purified enzyme (see below).

T4 mutants of genes 39, 52 and 60 eliminate topoisomerase activity

A large number of T4 mutants were screened for the ability to affect or abolish the ATP-dependent DNA topoisomerase activity seen in Fig. 1. For this purpose, sonically disrupted cell extracts were prepared from a variety of T4 mutant-infected cells. The mutants screened for activity are listed in Table 1; of these only *amN116* (gene 39⁻), *amH17* (gene 52⁻) and *amHL626* (gene 60⁻) showed no detectable DNA topoisomerase activity in extracts. As expected, the activity was missing when these amber mutant phages were used to infect a non-suppressing (*su*⁻) *E. coli* host (*E. coli* B); activity was present when the same mutants were used to infect an amber-suppressor strain (*E. coli* CR63). Furthermore, when any two of these three amber mutants were used to co-infect the non-suppressing *E. coli* host (*E. coli* B), the topoisomerase activity was largely recovered (see Table 1). Thus, the genetic complementation observed between mutants in these three genes *in vivo* is matched by activity complementation for the

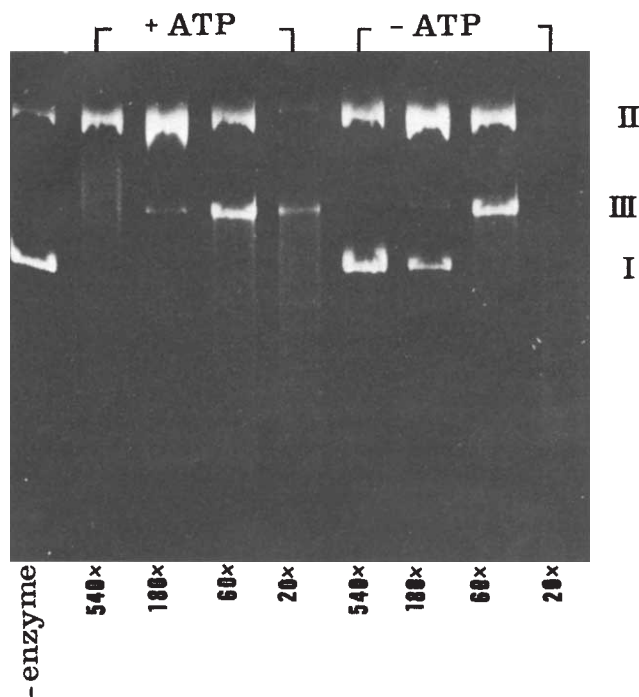


Fig. 1 An ATP-dependent DNA topoisomerase activity can be detected in extensively diluted crude extracts of T4 bacteriophage-infected *E. coli*. The Roman numerals on the right-hand margin indicate the positions after agarose gel electrophoresis of form I (covalently closed, negatively supercoiled duplex DNA circle), form II (nicked DNA circle) and form III (unit-length linear) PM2 DNA molecules. A completely relaxed closed circular DNA would co-migrate with form II DNA on this gel. The bands migrating between form II and form III DNAs in the second lane from the left (adjacent to the no enzyme control) are the covalently closed DNA circles which have a reduced superhelical density due to topoisomerase action. Note that cleavages by endogenous nucleases produce form II and form III DNAs at high extract concentrations preventing the detection of the topoisomerase activity. Reaction mixtures (20 μ l each) contained 50 mM Tris-HCl, pH 7.8, 40 mM KCl, 25 mM MgCl₂, 0.5 mM dithiothreitol, 0.5 mM Na₃EDTA, 30 μ g ml⁻¹ human serum albumin, 25 μ g ml⁻¹ covalently closed circular PM2 DNA and 0.5mM ATP (where indicated). To this mixture, 1 μ l of extract diluted to various extents was added to start the reaction; the numbers below each gel lane represent the final dilution of the original extract in the 20- μ l reaction. After 30 min at 30 °C, each reaction was stopped by adding 5 μ l of 50% glycerol, 5% SDS and 1 mg ml⁻¹ bromophenol blue and loaded on a 0.7% agarose gel. The gel was electrophoresed at room temperature in a buffer consisting of 90 mM Tris-borate (pH 8.3) and 5 mM MgCl₂ for about 15 h at 2.5 V cm⁻¹. To visualise the DNA, the gel was stained with 1 μ g ml⁻¹ ethidium bromide, visualised on a short-wave UV transilluminator and photographed with Polaroid film type 55. The source of infected cells for these experiments was *E. coli* strain D110 (thy⁻, polA⁻, endI⁻), which had been infected for 30 min at 37 °C with a MOI of 10 of T4 bacteriophage [*regA*-*amN55*(42⁻)-*amH39*(30⁻)] in M9 minimal medium supplemented with 0.3% casein hydrolysate, 1 μ g ml⁻¹ thiamine and 10 μ g ml⁻¹ thymidine. The infected cells (5 $\times$ 10⁸ cells ml⁻¹) were chilled, washed and concentrated 800-fold by centrifugation. After Brij lysis²⁹, the lysate was centrifuged at high speed (60 min at 35,000g), and the supernatant was used as the extract.

topoisomerase in extracts. Note, however, that the identification of the gene 60 product as being involved here is made less clear by complications arising from *E. coli* B-*E. coli* K host-range differences (see refs 4, 5).

Because infection of *E. coli* B with T4 amber mutants in genes 39, 52 or 60 causes a major delay in DNA synthesis, they have been designated as 'DNA-delay' (DD) mutants¹⁰. DNA-delay amber mutants infecting su⁻ *E. coli* eventually produce a substantial burst of progeny phage, although reduced relative to wild-type infection. This suggests that growth of the DNA-delay mutants on a su⁻ *E. coli* depends on a host function which is only

partially effective⁴. Using drug inhibition studies, McCarthy recently demonstrated that one compensating host factor is *E. coli* DNA gyrase, as, unlike wild-type T4, the growth of T4 gene 39, 52 and 60 mutants is very sensitive to the DNA gyrase inhibitors coumermycin and novobiocin^{11,12}. Using autoradiography to measure the replication fork rates during gene 52 mutant infections, McCarthy *et al.*⁶ have further suggested that the DNA-delay mutants are defective only in the initiation of T4 DNA replication, as the fork rates observed are normal.

Table 1 T4 mutants in genes 39, 52 and 60 eliminate the T4 DNA topoisomerase activity

T4 mutant	Relative T4 DNA topoisomerase activity	
	Su ⁻ <i>E. coli</i>	Su ⁺ <i>E. coli</i>
Uninfected	<5	<5
<i>amH39</i> (30 ⁻)	(100)	(100)
<i>amN116</i> (39 ⁻)	<5	25
<i>amH17</i> (52 ⁻)	<5	25
<i>amHL626</i> (60 ⁻)	<5	100
<i>amN116</i> (39 ⁻) + <i>amH17</i> (52 ⁻)		
co-infection	20	NT
<i>amH17</i> (52 ⁻) + <i>amHL626</i> (60 ⁻)		
co-infection	15	NT
<i>amHL626</i> (60 ⁻) + <i>amN116</i> (39 ⁻)		
co-infection	30	NT

Both *E. coli* CR63 (su⁺) and *E. coli* B (su⁻) were grown at 37 °C in 20 ml of H broth to a cell density of 5 $\times$ 10⁸ cells ml⁻¹; each culture was then infected at a MOI of 10, for each of the indicated T4 bacteriophages. At 12 min after infection, the cells were chilled, pelleted and resuspended in 320 μ l of 50 mM Tris-HCl, pH 7.8, 50 mM KCl, 10 mM β -mercaptoethanol and 1 mM Na₃EDTA. Each batch of cells was broken by sonication and centrifuged for 15 min at 8,000g in an Eppendorf model 3200 centrifuge. Serial dilutions of each supernatant were then assayed as described in Fig. 1 legend, except that 0.2 mg ml⁻¹ naladixic acid was present in each assay. The activity in each extract was estimated by comparing the relaxation activity with that in serial dilutions of the T4-*amH39* (gene 30⁻, DNA ligase) infected *E. coli* B extract, whose activity was taken as 100. Other mutants tested, with activities comparable to wild type were *amN134*-*amBL292*-*amE219* (33⁻, 55⁻, 61⁻), SP62-*amN55*-*amH39* (*regA*⁻, 42⁻, 30⁻), *amHL628* (59⁻), *amN011* (47⁻), *amN130* (46⁻)-*amE727* (49⁻), and the large non-essential deletions: Δ (far P13), Δ (63-32) and Δ (39-56)₁₂-alc⁻- Δ Sa Δ 9(ac-denB)-nd28(denA). NT, not tested.

Purified T4 DNA topoisomerase has multiple components

Starting from 100 g of T4-infected *E. coli* cells, 0.8 mg of highly purified T4 DNA topoisomerase was obtained following the multi-step procedure given in Fig. 2 legend. The total purification, estimated from specific activity measurement, was 350-fold, with a yield of 5%. With a unit of activity defined as the amount of enzyme necessary to induce half-relaxation of 0.3 μ g of pBR322 DNA in 30 min at 30 °C in our standard reaction mixture (see Fig. 1 legend), the final DNA-cellulose-purified T4 DNA topoisomerase had a specific activity of 10⁶ units per mg. As revealed by the Coomassie-stained SDS-polyacrylamide gel shown in Fig. 2, this fraction contains two major protein bands (molecular weights 63,000 and 52,000), which comprise about 70% of the total stained protein in the gel. Based on previous molecular weight estimates¹³, these two bands are almost certainly the products of T4 genes 39 (MW assigned at 64,000) and 52 (MW assigned at 51,000), respectively. Two other protein bands with MWs of 23,000 and 16,000 are also present on our gels. Of these two species, only the 16,000-MW protein actually co-purifies with the topoisomerase activity (data not shown). In view of the data presented in Table 1, and the genetic evidence that the gene products of 39, 52 and 60 interact with each other *in vivo*, it is tempting to identify the 16,000-MW protein as the T4 gene 60 product.

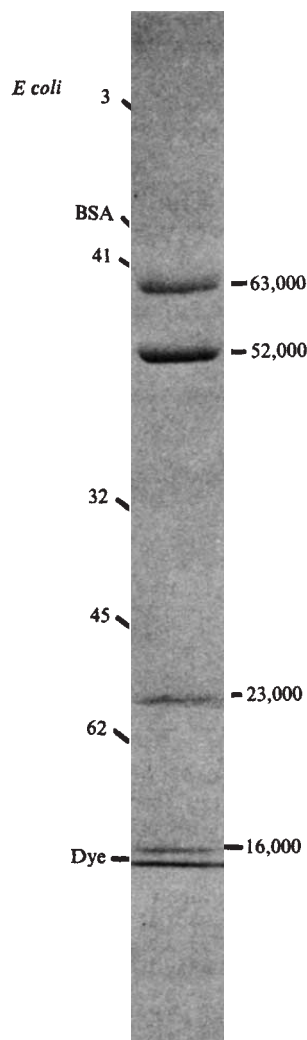


Fig. 2 SDS-polyacrylamide gel analysis of highly purified T4 DNA topoisomerase. A total of 9 μg of purified T4 DNA topoisomerase was denatured in SDS and electrophoresed on a 10% polyacrylamide slab gel³⁰; the resulting protein bands were then stained with Coomassie blue as described elsewhere³¹. The approximate polypeptide MWs observed are indicated on the right hand margin, and the migration positions for other purified T4 replication proteins^{29,32} used as markers (listed by T4 gene numbers), and for the *E. coli* ω protein⁷, are indicated on the left hand margin. To purify the enzyme, frozen T4 (*regA-amN55-amH39*) infected *E. coli* D110 cells (100 g) were thawed and resuspended in 150 ml of buffer containing 40 mM Tris HCl, pH 7.8, 2 mM Na_3EDTA , 25% sucrose and 95 mg of egg-white lysozyme (Worthington). After 60 min at 0°C, the cells were lysed by adding 150 ml of 1% (w/v) Brij 58, 40 mM Tris-HCl and 20 mM β -mercaptoethanol and stirring at 0°C for another 60 min. After centrifugation at 30,000g for 1 h, the 330 ml of supernatant was loaded on to a DEAE-cellulose (Worthington DE-52) column (2.5 cm $\times$ 24 cm) equilibrated with buffer A (40 mM Tris HCl, pH 7.8, 10 mM β -mercaptoethanol, 1 mM Na_3EDTA and 10% (w/v) glycerol). The flow-through fractions were pooled and precipitated by gradual addition of 10% (w/v) polymin P solution (pH 7.9) to a final concentration of 0.3%. The precipitate was collected by centrifugation (23,000g for 15 min), and the topoisomerase activity was then solubilised by extraction with 100 ml of 0.2 M NaCl (in buffer A). The topoisomerase was precipitated by ammonium sulphate (50 g per 100 ml), collected by centrifugation and redissolved in 30 ml of buffer A. After dialysis overnight against 2 l of buffer A, the topoisomerase was loaded on to a second DE-52 column (2.5 cm $\times$ 8 cm) equilibrated with buffer A. The flow through from this column was loaded directly on to a hydroxyapatite column (2.5 cm $\times$ 8 cm) equilibrated with solution B (10% glycerol, 10 mM β -mercaptoethanol) containing 20 mM potassium phosphate (pH 7.2). After washing the column with solution B containing 0.3 M potassium phosphate, the topoisomerase activity was eluted with solution B containing 0.5 M potassium phosphate. Pooled fractions with activity were dialysed into buffer A containing 50 mM KCl and loaded at 4 ml h⁻¹ on to a DNA-cellulose column (1.2 cm $\times$ 3 cm containing 1 mg per packed ml of single-stranded calf thymus DNA). This column was eluted successively with buffer A containing 50 mM, 100 mM, 200 mM and 300 mM KCl. The topoisomerase appeared in the 200 mM KCl eluate. The peak activity was pooled and dialysed against a buffer consisting of 30 mM potassium phosphate (pH 7.2), 50% (w/v) glycerol, 10 mM mercaptoethanol and 0.5 mM Na_3EDTA , for storage at -20°C, at which it seems to be quite stable.

The purified T4 DNA topoisomerase can be shown to act catalytically: one enzyme molecule (assumed to have a MW of 131,000) is capable of relaxing to completion at least seven molecules of pBR322 DNA in a 30-min incubation. The topoisomerase activity is completely resistant to novobiocin (up to 60 $\mu\text{g ml}^{-1}$), but is weakly sensitive to high concentrations of nalidixic acid. This inhibitory effect of nalidixic acid was further studied by using oxolinic acid, a more potent analogue of nalidixic acid; the T4 DNA topoisomerase activity was halved by oxolinic acid at about 250 $\mu\text{g ml}^{-1}$. As this level of oxolinic acid is about an order of magnitude higher than the concentrations needed for the inhibition of *E. coli* DNA gyrase^{14,15}, it is likely that the observed inhibition is nonspecific. A similar 'nonspecific' inhibition has been previously observed for another DNA topoisomerase, *E. coli* ω protein, at comparably high oxolinic acid concentrations (500 $\mu\text{g ml}^{-1}$, ref. 15). When tested with a variety of different circular DNA molecules, the topoisomerase activity shows no obvious DNA preference. The purified enzyme exhibits no DNA gyrase activity with either fully relaxed pBR322 DNA or PM2 DNA in our assay conditions (that is, it is unable to introduce supercoils into these DNA molecules).

Both in the crude extract and in the first DEAE-cellulose breakthrough, the topoisomerase activity is associated with a 30S complex, as judged by sucrose gradient sedimentation (data not shown). This complex can be destroyed, without affecting the ATP-dependent relaxation activity, either by pancreatic RNase A treatment or by salt-extracting the enzyme from a precipitate formed with the synthetic polycation polymin P, as described in Fig. 2 legend. The significance of this apparent RNA association in crude fractions is not known. However, our purified enzyme contains less than 1% nucleic acid, and it sediments more slowly than 5S.

The topoisomerase can catalyse the ATP-dependent relaxation of positive DNA twists

Unlike the *E. coli* ω protein (which can only remove negative turns), T4 DNA topoisomerase catalyses the relaxation of both negatively and positively superhelical DNAs. To assay for the relaxation of positively supercoiled DNA, a fully relaxed PM2 DNA substrate was prepared, and ethidium bromide was added to make this relaxed DNA act as if positively supercoiled. The relaxation of the positive DNA twists present in these conditions can be detected by the increased mobility of the covalently closed PM2 DNA, seen when this DNA is subjected to electrophoresis in the absence of ethidium bromide (see Fig. 3a). With our highly purified T4 DNA topoisomerase, we have also demonstrated that: (1) the relaxation of both negative and positive twists is ATP dependent; (2) both reactions are gradual, indicating that the enzyme does not relax the DNA by a one-hit mechanism and that it dissociates from each DNA molecule before relaxing it completely, (3) the enzyme acts catalytically in both cases, in that one T4 DNA topoisomerase molecule can relax many PM2 DNA molecules in a 30-min reaction (data not shown).

ATP hydrolysis is required for catalytic relaxation

The relaxation of superhelical DNAs of both senses is a thermodynamically favourable process. Correspondingly, none of the relaxation reactions catalysed by the other known DNA topoisomerases requires an exogenous source of energy⁷. In contrast, we have shown (Fig. 1) that the T4-induced DNA topoisomerase requires ATP as a cofactor. The requirement for ATP cannot be replaced by other ribo- or deoxyribonucleoside

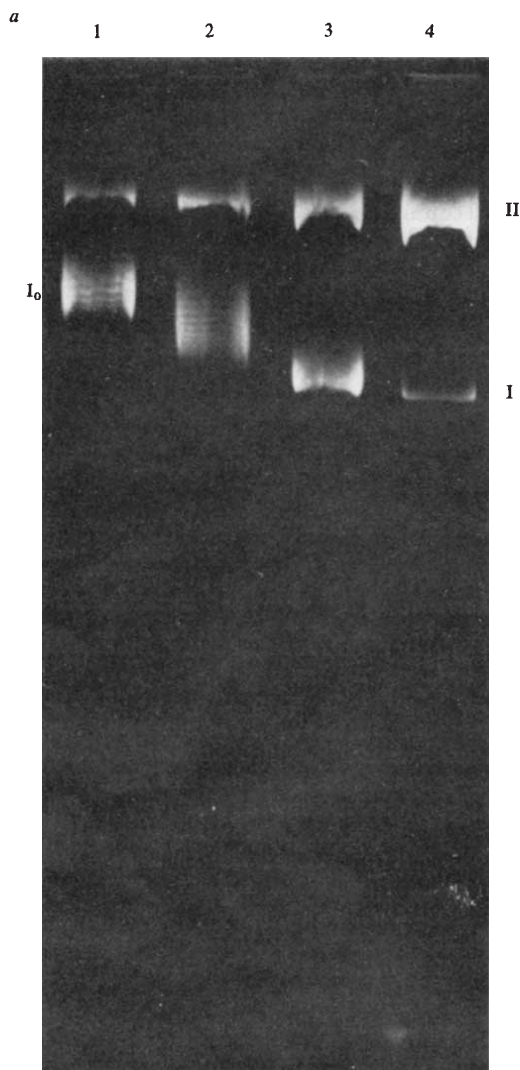
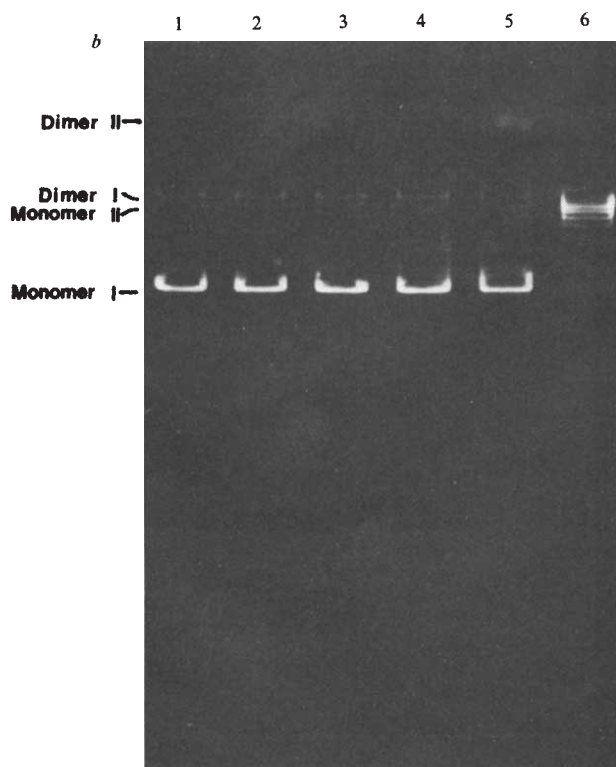


Fig. 3 *a*, The ATP-dependent relaxation of positively superhelical PM2 DNA catalysed by the T4 DNA topoisomerase. Reactions were carried out as described for Fig. 1, except that fully relaxed, covalently closed PM2 DNA (form I_0) was the substrate and ethidium bromide was present at $2 \mu\text{g ml}^{-1}$ to induce positive superhelical turns³³. As the source of the topoisomerase, the breakthrough of the initial DE-52 chromatography step (described in Fig. 2 legend) was used. This partially purified topoisomerase was diluted into the reaction mixture as follows: lane 1, no enzyme; lane 2, 2,000-fold; lane 3, 500-fold; lane 4, 100-fold. Electrophoresis was carried out as described for Fig. 1, but at 4°C . The removal of ethidium bromide during electrophoresis causes the unreacted substrate DNA to return to a relaxed conformation, but a small amount of negative superhelicity is induced by the low temperature used for electrophoresis. As a result, at 4°C , the substrate DNA (denoted as form I_0) migrates faster than nicked circular DNA (form II), but more slowly than the highly negatively supercoiled form I DNA. In contrast, the DNA relaxed in the presence of ethidium bromide gains a great deal of negative superhelicity on removal of this dye; thus, the final product of the topoisomerase reaction is the rapidly migrating form I DNA. Note that, with increasing enzyme, increasing numbers of the covalently closed DNA circles which survive the incubation; this change in superhelicity is only obtained when ATP is present (data not shown). In addition, the topoisomerase still contains an endonuclease contaminant at this stage, which causes the amount of form II DNA to increase as more enzyme is added. *b*, When inducing DNA relaxation, the topoisomerase acts catalytically and requires ATP hydrolysis. Reaction mixtures ($20 \mu\text{l}$ each) contained 50 mM Tris-HCl, pH 7.8, 60 mM KCl, 10 mM MgCl_2 , 0.5 mM dithiothreitol, 0.5 mM Na_3EDTA , $30 \mu\text{g ml}^{-1}$ human serum albumin, $20 \mu\text{g ml}^{-1}$ form I pBR322 DNA (negatively supercoiled) and $0.2 \mu\text{g ml}^{-1}$ of the most highly purified fraction of T4 DNA topoisomerase. Incubations were carried out for 30 min at 30°C , and the products were analysed as described for Fig. 1. Lane 1 is the no enzyme control; lanes 2–6 represent reactions carried out with $70 \mu\text{M}$ rATP present, plus different amounts of the non-hydrolysable ATP analogue, ATP- γS , as follows: lane 2, 500 μM ; lane 3, 200 μM ; lane 4, 50 μM ; lane 5, 10 μM ; lane 6, no ATP- γS . As nearly complete inhibition of the topoisomerase activity occurs with 10 μM ATP- γS in the presence of $70 \mu\text{M}$ ATP (lane 5), it is clear that the K_i for ATP- γS is much lower than the K_m for ATP.



triphosphates. This surprising energy requirement has prompted us to investigate the ATP dependence further.

The fact that actual ATP hydrolysis (rather than merely the presence of ATP) is required comes from two lines of evidence. First, the compound rATP- γS [riboadenosine 5'-O-(3-thiotriphosphate)] inhibits the topoisomerase reaction strongly when added to our normal reaction mix, as shown in Fig. 3b. rATP- γS is an analogue of rATP which is poorly hydrolysed by enzymes which cleave the β - γ phosphodiester linkage of ATP. It acts as an inhibitor of many ATPases, including the activities of the T4 gene 41 and 44/62 proteins (unpublished observation of this laboratory). Second, as shown in Table 2, a DNA-dependent ATPase activity can be detected in the most purified fraction of T4 DNA topoisomerase, for which the hydrolysis products are ADP and inorganic phosphate. With certain assumptions (see Table 2 legend), we can calculate that the number of ATP molecules hydrolysed is roughly equal to the number of superhelical DNA turns relaxed by the topoisomerase.

It is important to note that although rATP- γS is a potent inhibitor of the T4 DNA topoisomerase-catalysed relaxation reaction, it can, nonetheless, stimulate the relaxation reaction in the absence of ATP when the enzyme is present at substrate levels (C.-C.L., unpublished results). This situation is analogous to the effect of a non-hydrolysable analogue of ATP, App(NH)p, on *E. coli* DNA gyrase¹⁶. For gyrase, the binding of ATP seems to induce a conformational change in the protein which leads to a single cycle of DNA supercoiling; the hydrolysis of ATP is then required to return the enzyme to its original conformation¹⁶. The situation for the T4 DNA topoisomerase is probably similar, except that one cycle of DNA relaxation,

Table 2 DNA-dependent ATPase activity of the T4 DNA topoisomerase

	ATP hydrolysed (nmol ml ⁻¹)		
	5 min	10 min	15 min
-DNA	<0.2	<0.2	0.2
+35 µg ml ⁻¹ ss fd DNA	0.9	1.9	2.5
+20 µg ml ⁻¹ pBR322 DNA	2.0	4.0	5.0
+50 µg ml ⁻¹ T4 ds DNA	3.7	8.3	10.5

The reaction mixtures (20 µl each) were as described in Fig. 1 legend, except that 100 µM [γ -³²P]ATP was present. The most purified T4 DNA topoisomerase (0.5 µg) was added to each reaction mixture. After incubation at 30 °C for the times indicated, aliquots (50 µl) of reaction mixtures were withdrawn and added to 0.5 ml of 0.1 M HCl. Hydrolysis was measured as inorganic ³²P (as phosphate) separated from unhydrolysed [γ -³²P]ATP by charcoal adsorption²⁷. The different DNAs present in each reaction mixture are indicated. That the products of hydrolysis are ADP and inorganic phosphate was determined in a separate experiment in which ³H-ATP was used as substrate and the products were analysed by chromatography on PEI-cellulose²⁸. From the ATP hydrolysis observed during a 30-min incubation at 30 °C using 0.5 µg of enzyme and 100 µM ATP, we calculate that about 1 pmol of ATP would be hydrolysed by 2.5 ng of enzyme (assuming that the dose response is linear for ATPase activity). In the same conditions, 2.5 ng of enzyme will relax about half of the superhelical turns in 0.1 pmol (as DNA molecules) of pBR322 DNA. As there are about 30 superhelical turns in each pBR322 DNA molecule in our reaction conditions, we estimate that one or two superhelical DNA turns are relaxed for every ATP molecule hydrolysed.

rather than DNA supercoiling, is observed on ATP binding. However, we cannot rule out the possibility that the stimulation is due to a very slow hydrolysis of ATP- γ S by the topoisomerase.

Similarities between the T4 DNA topoisomerase and *E. coli* DNA gyrase

At least two kinds of DNA topoisomerase are found in bacteria, as exemplified by the *E. coli* ω protein (*Eco* DNA topoisomerase I) and the *E. coli* DNA gyrase (*Eco* DNA topoisomerase II). *E. coli* ω protein catalyses the energetically favourable partial relaxation of negatively superhelical DNA with no added energy cofactor¹⁷. In contrast, DNA gyrase³⁶ catalyses the reverse reaction, introducing negative superhelicity into DNA in a reaction requiring ATP hydrolysis^{16,18}.

The T4 topoisomerase and the *E. coli* gyrase are multiple-subunit proteins which have both nicking-closing and DNA-dependent ATPase activities. In both cases, these two activities are apparently tightly linked, inasmuch as each cycle of nicking and closing needs to be accompanied by hydrolysis of one (or two) ATP molecules^{16,18}. Finally, for both enzymes addition of SDS and protease seems to trap nicked DNA-enzyme intermediates, in which both DNA strands have been broken to produce a unit-length linear DNA molecule from a double-stranded DNA circle (refs 14, 15 and L.F.L., unpublished results). Nevertheless, there is at least one important difference: only the *E. coli* gyrase is able to harness its ATP hydrolysis energy to do useful work (induce DNA supercoiling); so far in our experiments the T4 DNA topoisomerase seems to be wasting this energy, as the reaction catalysed is already thermodynamically favourable without ATP hydrolysis.

How might T4 DNA topoisomerase function in replication fork initiation?

The replication machinery of T4 bacteriophage has largely been reconstructed *in vitro*. Using seven highly purified T4 proteins essential for T4 DNA replication *in vivo*, DNA synthesis has been demonstrated to occur efficiently and with high fidelity on double-stranded DNA templates¹⁹. However, this synthesis begins by a strand displacement reaction which is initiated by covalent addition on to the 3'-end of a pre-existing DNA nick¹⁹. The expected *de novo* fork initiation, by formation of an *in*

vivo-like 'replication bubble' on a double-stranded T4 DNA molecule, has not been achieved in this (or any other) purified *in vitro* system. The previously described genetic evidence implying that the T4 gene 52, 39 and 60 products are specifically involved in T4 replication fork initiation, coupled with the present identification of these proteins as a topoisomerase, suggests that this enzyme may be the missing factor.

The tight coupling between the ATP hydrolysis and relaxation activities of T4 DNA topoisomerase is unique among all known DNA topoisomerases. Although it is possible that the ATP hydrolysis functions directly in the nicking-closing reaction (for example, by being used to provide energy for ligation of the putative broken DNA chain intermediates), it seems much more likely that the ATP hydrolysis energy is normally coupled to another, as yet unidentified, energy-utilising reaction.

We have no direct evidence as to how ATP energy is used by the topoisomerase at the replication origin. However, we will present a speculative model which is suggested by the above-mentioned similarities between the T4 DNA topoisomerase and DNA gyrase. In this view, the T4 DNA topoisomerase serves as a DNA gyrase-like enzyme which acts specifically at the T4 replication origin. Such an enzyme might function to initiate new T4 replication forks in a manner analogous to that schematically outlined in Fig. 4. Here, the T4 DNA topoisomerase would recognise two specific DNA sequences spanning the origin²⁰ of T4 DNA replication. By anchoring these two DNA sequences on the same enzyme molecule and thereby restricting the rotation of the DNA strands relative to the enzyme, the topoisomerase could create a closed topological domain. This would allow its hypothetical ATP-driven gyration to induce a local negative supercoiling which destabilises the double helix at the replication origin. As indicated, the melting of this origin would presumably require the gene 32 helix-destabilising protein. Participation of the T4 gene 41 and/or gene 44/62 replication proteins, destabilising the helix by ATP-driven walking

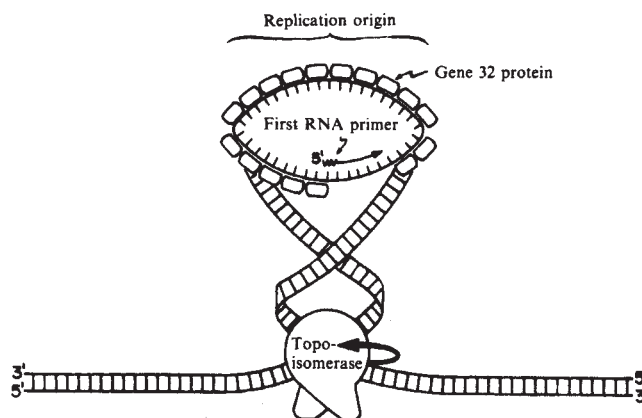


Fig. 4 Two possible models for T4 DNA topoisomerase action at the T4 replication origin. These two models assume (without proof) that the rate-limiting step in generating new replication forks is the opening of the DNA helix at the origin (see text). *a*, Topoisomerase gyration. In this hypothetical scheme, the topoisomerase acts as a site-specific DNA gyrase and uses its ATP hydrolysis and nicking-closing activities to separate the strands of the DNA helix at the T4 replication origin. The replication origin must form a closed loop, as an isolated domain is required in the otherwise linear T4 DNA molecule to allow supercoiling. This loop could be formed either by the binding of the topoisomerase alone (as indicated here) or by a separate set of proteins or membrane sites. The T4 gene 39 and 52 proteins have been reported to interact with the cell membrane, as well as with DNA³⁴, and so some membrane attachment might also be used to constrain the DNA origin in this way. (It is interesting that limited topological domains have been detected in the condensed T4 chromosome³⁵.) In any case, the topoisomerase is suggested to recognise a set of specific DNA sequences which designate a T4 chromosome origin, and to show a DNA gyrase-like activity there (induction of negative twists which destabilise the helix) which is not detected with the non-origin DNA sequences present in our standard topoisomerase assay.

along DNA single-strands^{21,22}, is also possible. Note that, in this view, the ATP-dependent relaxation of non-origin-containing DNA molecules observed *in vitro* reflects an uncoupled version of a site-specific DNA gyration reaction catalysed by the topoisomerase *in vivo*.

The site-specific gyration model in Fig. 4 postulates that the initial opening of the DNA helix is followed by an RNA-primed DNA chain start, which actually begins DNA synthesis. This first RNA primer could either be synthesised by the same enzymes that prime the Okazaki pieces made on the lagging strand of the T4 replication fork, or be made by a separate mechanism^{3,23}. It has been shown recently that the T4 gene 61 protein, in conjunction with the T4 gene 41 protein, can synthesise pentaribonucleotides, and that this synthesis has an absolute requirement for a single-stranded DNA template (ref. 19 and C.-C.L., unpublished results). When additional replication proteins are present, these short ribonucleotides will prime lagging strand DNA synthesis in the T4 *in vitro* system¹⁹. These two T4 proteins may therefore also have a crucial role in replication fork initiation.

Despite our limited knowledge concerning the mechanism of initiation of DNA replication forks, some similarities can be seen between different systems. As is the case for many other genomes (bacteriophage λ , *E. coli* and eukaryotic chromosomes), replication forks in T4 seem to be able to start out bi-directionally from an initial replication bubble²⁴. We therefore expect that a detailed study of the T4 DNA topoisomerase and its interaction with the other T4 replication proteins can provide a model system in which some general properties of fork initiation mechanisms can be elucidated.

Genetic data suggest that genes *O* and *P* of bacteriophage λ may act in a manner analogous to T4 DNA topoisomerase. As for T4 genes 39, 52 and 60, the λ *O* and *P* products interact with each other, and when their levels are decreased, the initiation of λ DNA replication is altered²⁵. Experiments comparing various lambdoid phages have shown the gene *O* product is highly phage specific, presumably because it recognises origin DNA sequences²⁶. Similarly, unlike most T4 genes, gene 39 function cannot be complemented by bacteriophage T2 (W. M. Huang, personal communication); this suggests that gene 39 may have a role in T4 replication which is analogous to the role of gene *O* in λ replication.

Further study of the T4 DNA topoisomerase should also provide valuable knowledge concerning the functions and mechanisms of the important class of enzymes known as DNA topoisomerases. In this respect, we have observed that, at much higher enzyme concentrations (about equal weights of DNA and T4 DNA topoisomerase), a novel ATP-independent DNA topoisomerisation reaction can be detected, in which topologically knotted, double-stranded superhelical DNA molecules are created. Most strikingly, these knots can subsequently be removed by catalytic amounts of the topoisomerase, in an efficient ATP-dependent reaction. As will be described in detail elsewhere, such data reveal that the T4 DNA topoisomerase is working by making transient double-stranded DNA breaks which are rapidly and efficiently resealed in its 'closing' reaction.

This work was supported by USPHS grant GM24020 from the National Institute of General Medical Sciences to B.M.A. and an American Cancer Society postdoctoral fellowship to L.F.L.

Received 21 May; accepted 30 August 1979.

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Erratum

In the letter 'Basaltic pillars in collapsed lava-pool on the deep ocean floor' by J. Francheteau *et al.*, *Nature* **281**, 209-211, the third author's name was mis-spelt; it should read C. Rangin. Figures 1 and 2 were incorrectly reproduced. They are shown correctly below.



Fig. 1 General landscape: pillars with collapsed lava pond.

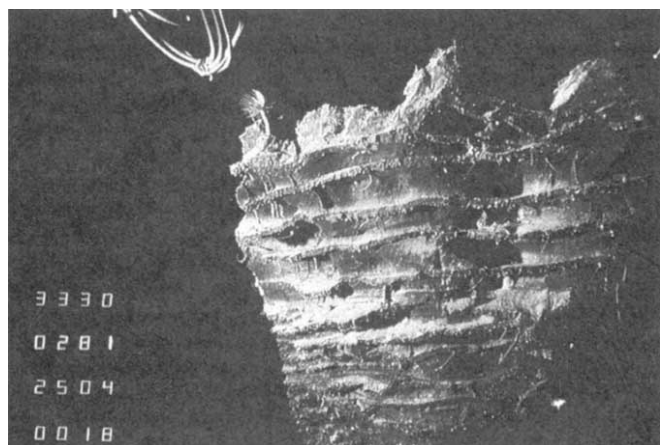


Fig. 2 Detail of a pillar showing the centimetric pseudo layering with thin and darker salient glass layers projecting from the basaltic surface of the pillar.

letters

HEAO 1 observations of 4U1232+07

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While performing an analysis of the NRL HEAO 1 observations of the Virgo cluster and 3C273, we detected X-ray emission from a region between these two objects. The most satisfactory explanation is the presence of two weak X-ray sources, for which we have obtained error boxes. One source is likely to be the peculiar galaxy IC3576, suggested by Margon *et al.*¹ as the source 2U1231+07=4U1232+07 (ref. 2). The error box resulting from combining our observations with the 4U data strengthens the X-ray identification with this extremely interesting galaxy. There are numerous possibilities for the other X-ray source, and here we discuss one, the interacting pair of galaxies NGC4410a and NGC4410b (NGC4410a/b).

The field of view of the X-ray detectors is $4^\circ \times 1^\circ$. The data we used were obtained from the superimposition of parallel scans within $\pm 0.75^\circ$ of the arc defined by $\alpha = 12\text{ h } 50.5\text{ min}$, $\delta = 16.2^\circ$ to $\alpha = 12\text{ h } 17.5\text{ min}$, $\delta = -1.6^\circ$. The relationship of the $4^\circ \times 1^\circ$ field of view to the scan, and the sky position of several objects is shown in Fig. 1. The average number of counts per 0.5° bin are plotted in Fig. 2. The data have been fitted to a diffuse source model for the Virgo cluster and two point sources for the region between 348 and 352 deg. The 3C273 data were not included in these fits. The least squares fit centres the Virgo cluster at scan

angle = 5.9° and the two point sources at scan angles 9.3° and 10.5° , as shown in Fig. 2. The region between 9 and 13 deg is fitted equally well by a diffuse source of core radius 176 arc min, but it is unlikely that a diffuse source so large would be in this region. This region is $\geq 7^\circ$ from the Virgo Cluster centre or M87, and, hence, is unlikely to be an extended region of hot gas within the Virgo Cluster. Also, there are no rich clusters or super-clusters in this direction, and therefore we believe two point sources to be a more likely interpretation of the data.

The HEAO and Uhuru error boxes are shown in Fig. 3. The corners of the parallelogram lying in both boxes are given by: $\alpha = 12\text{ h } 35\text{ min } 30\text{ s}$, $\delta = 6^\circ 50'$; $\alpha = 12\text{ h } 31\text{ min } 10\text{ s}$, $\delta = 7^\circ 19'$; $\alpha = 12\text{ h } 35\text{ min } 07\text{ s}$, $\delta = 6^\circ 38'$; $\alpha = 12\text{ h } 30\text{ min } 36\text{ s}$, $\delta = 7^\circ 10'$; and the centroid of the error box is at $\alpha =$

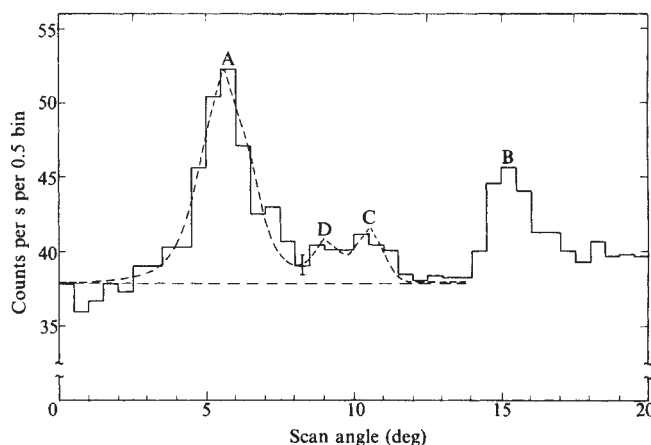


Fig. 2 The counts per s are plotted per 0.5° bin as a function of scan angle. The small dashes show the fit to a diffuse source model for the Virgo (A) and two point sources (C and D) for the region beyond. The source B is 3C273. The dashed horizontal line represents the background emission.

$12\text{ h } 33\text{ min } 06\text{ s}$, $\delta = 6^\circ 59.2'$. The coordinates of IC3576 are $\alpha = 12\text{ h } 34\text{ min } 5.4\text{ s}$ and $\delta = 6^\circ 53' 47''$. The area of the parallelogram is 0.25 deg^2 . We conclude that the identification suggested by Margon *et al.*¹ is correct. IC3576 is listed as a dwarf galaxy by van den Berg⁴, but this is doubtful (see Margon *et al.*¹). It has been studied in detail at optical and radio¹ wavelengths, and we summarise these results as follows: (1) its magnitude is 15.2 (ref. 5). The galaxy has no well-defined spiral structure and has several blue condensations which might be OB associations; (2) radio observations of the galaxy show a very unusual ratio of neutral hydrogen to optical luminosity, the ratio being almost 10 times greater than that for a normal galaxy and almost twice that for a dwarf galaxy. Its distance is about 20 Mpc¹, and therefore its X-ray luminosity (1–10 keV) is $10^{42}\text{ erg s}^{-1}$. This is about 500 times stronger than that for our own galaxy. IC3576 is clearly worth further investigation.

The centroid for the other X-ray object is $\alpha = 12\text{ h } 34\text{ min } 57.6\text{ s}$, $\delta = 8^\circ 11' 24''$. The corners of the 90% confidence error box are: $\alpha = 12\text{ h } 46\text{ min } 38.4\text{ s}$, $\delta = 7^\circ 18' 36''$; $\alpha = 12\text{ h } 45\text{ min } 12\text{ s}$, $\delta = 6^\circ 32' 24''$; $\alpha = 12\text{ h } 24\text{ min } 36\text{ s}$, $\delta = 9^\circ 49' 12''$; $\alpha = 12\text{ h } 23\text{ min } 9.6\text{ s}$, $\delta = 9^\circ 2' 24''$.

We found no QSOs (in the list by Burbidge *et al.*⁶) in the error box. There is one 4C object, 4C09.41 (ref. 7), just outside the error box, and the interacting pair of galaxies NGC4410a/b within the error box. Condon and Dressel⁸ have suggested that a

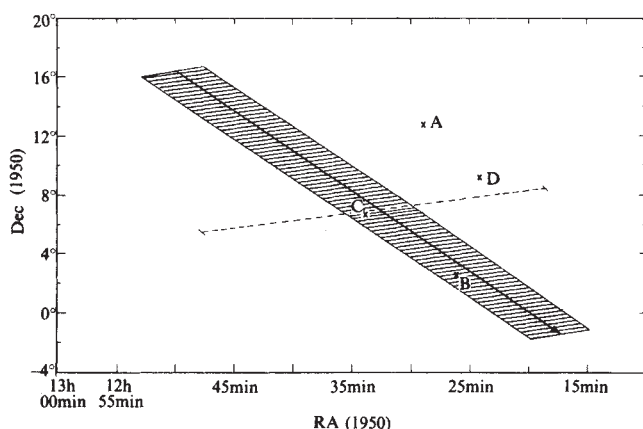


Fig. 1 The $\pm 0.75^\circ$ wide shaded region shows the portion of the sky covered by the centre of the field of the X-ray collimator over several scans. The arrow shows the direction of the scans for reference with Fig. 2. The dashed line ($\pm 4^\circ$) delineates the 4° direction of the X-ray collimator (see text). The positions of A, B, C and D are for M87, 3C273, IC3576 and NGC4410a/b, respectively.

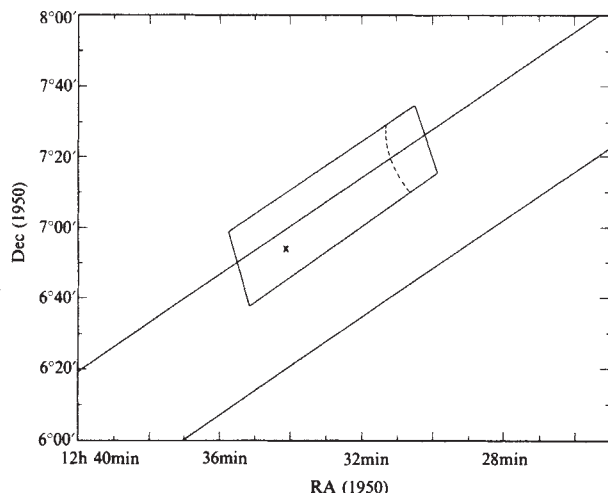


Fig. 3 The error box for 4U1232+07 is shown and superimposed on it is a portion of our error box (the two parallel lines). The dashed line, combined with the left hand portion (east) of the 4U error box is the allowed region for the source determined by Margon *et al.*¹. × represents the position of IC3576 on the sky.

quiescent galactic nucleus might be triggered into radioactivity by a close encounter between two galaxies, and Griffiths *et al.*⁹ have extended this idea to the X-ray range by suggesting that interacting pairs are likely to be X-ray sources. Schanberg¹⁰ has shown that the pair NGC4410a/b is indeed interacting, so that, for example, tidal distortion is evident in the outer regions of NGC4410a/b. If the system is an X-ray source, its luminosity is about $5 \times 10^{43} \text{ erg s}^{-1}$.

S.P.B. and M.P.U. thank staff of the NRL for hospitality and support during the data-reduction phase of this work. This research was supported, in part, by NASA contracts to Northwestern University and NRL.

Received 2 July; accepted 22 August 1979.

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Near IR variability of the quiet Sun

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The IR region of the solar spectrum provides important diagnostics for heat transfer processes in the photosphere, where the gravity and acoustic waves which heat the corona originate. Rates of molecule formation and dissociation in these layers are highly temperature sensitive. Recently relative intensities and short-period variations in individual lines of the rotation–vibration band of CO at $4.7 \mu\text{m}$ (refs 1–3) were obtained with spectral resolution $\nu/\Delta\nu \sim 0.7\text{--}2.1 \times 10^4$ (refs. 4, 5) from ground-based sites. High resolution cannot wholly overcome atmospheric absorption problems, so to measure absolute energy and variability in the band we have observed the solar spectrum from the orbital station Salyut 5.

The spectrometer for the region $1.8\text{--}15 \mu\text{m}$ used a bolometric detector and a Littrow–Pfund optical layout with an NaCl prism. It was fed by a newtonian telescope of 45 cm focal length with a $19 \text{ cm} \times 24 \text{ cm}$ primary, giving a field of view of $4 \text{ arc min} \times 24 \text{ arc min}$ as defined by the entrance slit. A typical recording time per spectrum is 4 s. The station served as an inertial observing platform, pointing to within an accuracy of a few arc min; stability was maintained via a solar imaging camera, with 2.5 s between images.

Of the spectra taken on 1, 4 and 5 August 1976 and 16 February 1977 we have chosen 46 from the east–south part of the disk, where independent data⁶ showed no solar activity such

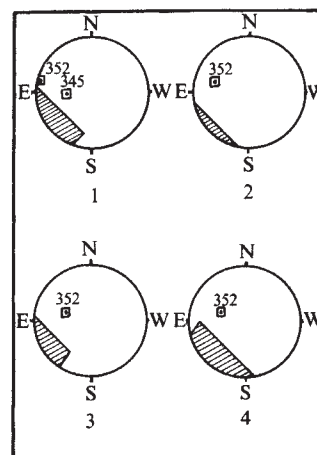


Fig. 1 The hatched areas show the regions on the solar disk on which the four groups of experiments were directed. □, The regions of solar activity during the experiments.

as spots or flares. The situation is illustrated in Fig. 1. Table 1 shows the timing of the selected groups of spectra.

Figure 2 shows the results of averaging each group of spectra in the wavelength range $4\text{--}6 \mu\text{m}$ where variability was observed. Between 4.05 and $5.25 \mu\text{m}$ there are excesses: for series 3 of 3%, for series 2 of 8% and for series 1 of 13% compared with the minimum curve of series 4. The r.m.s. error in the data is between $<1\%$ and 2% . An estimate of systematic error was obtained from data with the slit 3 arc min from the solar limb, when a signal value of 1% was registered. Guidance errors are 1 arc min maximum, and scattered light was minimised by diaphragms at three pupils: the spectrometer slit, the output slit and the bolometer. We can thus assert that the observed CO

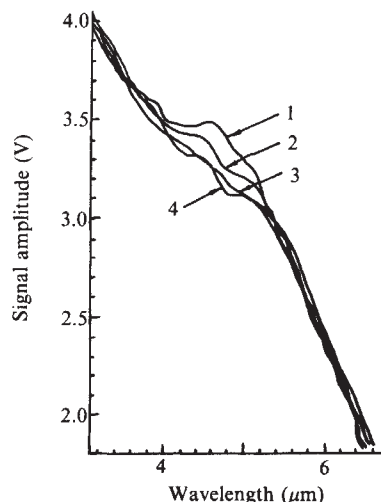


Fig. 2 Spectral dependence of a signal at the spectrometer output for the experimental groups 1, 2, 3 and 4.

Table 1 Timing of the 46 selected spectra

Series	Orbit no.	No. of spectra	Date (August 1976)	Time duration	$F_{\odot}(2,800 \text{ MHz})$ ($\text{Wm}^{-2} \text{ Hz}^{-1}$)
1	640	24	1	11 h 21 min 40 s–11 h 23 min 20 s	75.9×10^{-22}
2	685	6	4	7 36 35 – 7 37 0	82.5×10^{-22}
3	700	6	4	6 43 50 – 6 44 15	82.5×10^{-22}
4	701	10	5	7 23 45 – 7 33 25	84.2×10^{-22}

Timing relates to the spacecraft orbit, and thus bears no periodic relation to the period of solar rotation or activity.

variability is real, and intrinsic to the quiet Sun. Absence of solar activity is indicated by the steady values of the 2,800 MHz radio flux $F_{\odot}$ (2,800 MHz) shown in Table 1. Geomagnetic field perturbations were low (index $A_p \sim 4$ –5) and two small $H\alpha$ flares, shown as 352 and 345 in Fig. 1 do not lie near our observing regions. OSO 8 X-ray data and Mt Wilson magnetograms listed in ref. 6 also indicated no flare activity there.

From previous, resolved spectra of the solar CO band^{4,5} one can conclude that the species contributing to the radiative flux are H^- (at an effective temperature of 5,400 K (ref. 7)) and CO (with $T \sim 4,500$ –4,900 K). The linewidth to separation ratio for CO is 1:10 so the CO absorption cannot be $>10\%$ of the continuum in the range 4.05–5.25 μm . An observed variability of 5–10% suggests complete disappearance and re-formation of CO over periods of the order of a few hours. This could be due to convective cycling, in the granules, of hotter material from below.

The observed variability in flux is of the order of $1.3 \times 10^4 \text{ W m}^{-2}$, that is, some 2×10^{-4} of the total flux of solar radiation. Theoretical estimates for the energy flux contained in gravity waves lie close to $4 \times 10^4 \text{ W m}^{-2}$ with an acoustic flux of similar order. Thus CO formation and destruction may provide an important sink and source of energy for the maintenance of the acoustic, gravity, and magneto-acoustic waves responsible for coronal heating. Although details must await a more

thorough analysis, one can point to suggestive theoretical support for the hypothesis. Theoretical values⁸ for the non-radiative component of the energy flux are much greater than the combined fluxes due to X-ray and UV radiation and the solar wind; CO radiation could balance the energy equation. Photospheric thermal relaxation could be affected by the CO radiation, thus affecting the propagation of gravity waves. Concentration changes in CO can enhance photospheric convective instability by changing the radiative temperature gradient. Finally, note that further observations with greatly increased angular resolution could be used to make direct observations relating to the energetics of convective granulation.

We thank Dr J. E. Beckman (Queen Mary College, London) for his considerable help with rewriting this paper.

Received 23 May; accepted 17 August 1979.

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An estimate of the fine structure constant

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Quantum electrodynamics, despite its many successes, has left many questions unanswered. Why is electrodynamics so different from other interactions? Why do all particles have commensurate electric charges? Why is the value of the fine-structure constant what it is? Today, we have an apparently correct and essentially complete description of all of elementary particle physics in terms of a gauge theory¹. If the underlying gauge group is simple^{2,3}, all three elementary particle interactions—strong, weak, and electromagnetic—are parts of one unified theory. (The earlier attempt to synthesise all three interactions obtained both charge quantisation and baryon number violation. It was not based on a simple gauge group, nor did it incorporate conventional quantum chromodynamics.) Electromagnetism is no longer so unique. Apparent differences among the interactions reflect the pattern of spontaneous symmetry breakdown. Charge quantisation is forced on us: all fields have integer multiples of one-third the electron's charge^{2,3}; all observable particles have integer multiples. (We assume that colour is a truly hidden variable, that quarks are fractionally charged and never liberated. For another point of view, see ref. 4.) The fine-structure constant cannot be chosen arbitrarily. For unified theories in which the proton is unstable, we find an upper limit to its strength, $\alpha < 0.04$. We derive this result here and survey the context in which it arises.

The minimal gauge group which is required to describe all three interactions is $H = \text{SU}(3) \times \text{SU}(2) \times \text{U}(1)$, not a simple group. Three kinds of quantum fields are needed: gauge bosons, Higgs bosons, and fermions: the 12 gauge bosons include eight gluons (confined, hence not directly observable), three intermediate vector bosons (heavy, hence not yet observed), and the photon. At least one Higgs boson must survive as an observable particle at accessible mass^{5,6}.

Fermions come in families of 15 chiral fields apiece⁷. (We put the tau lepton and the up quark into a third 15-plet, also including a not-yet-seen $Q = 2/3$ quark.) One family—containing up and down quarks, electrons, and electron neutrinos—suffices for a description of most phenomena. There exist at least two additional families. We do not know why all families have the same format, nor why there are three families, nor whether there are more than three. (The $\text{SU}(3) \times \text{SU}(2) \times \text{U}(1)$ representation content of each of the families is identical. Each family is a minimal anomaly-free representation of H . This behaviour survives unification in its simpler forms. Each family is a reducible $10 + \bar{5}$ representation of $\text{SU}(5)$, or an irreducible spin representation of $\text{O}(10)$. In no plausible scheme do all three families belong to a single irreducible representation.) Each family is supposed to contain a weakly-coupled massless, or nearly massless, neutrino. The number of these that may exist is related to the observed helium abundance in the Universe^{8–10}, and not many more than three families are tolerable. On the other hand, three is the minimum number of families for which an elegant description of CP violation makes sense¹¹.

The theory based on the gauge group H and involving three fermion families is not a unified theory. It does not explain charge quantisation. It involves 17 independent dimensionless parameters¹², each as fundamental as the fine-structure constant. Although 17 free parameters must be too many for a

fundamental theory to admit, it should be considered that all elementary particle properties can be expressed (in principle) in terms of them. Perhaps the gauge group H is merely the phenomenologically-relevant remnant of a larger and simple unifying group G . The three independent gauge coupling constants are reduced to one, and quantisation of charge is assured. Restrictive hypotheses can yield further relationships among the 17 parameters¹³. Such theories have the following properties: (1) G has more generators than does H . They correspond to a fourth class of gauge boson which mediates new types of interaction. Generally, the new interactions leads to the decay of the proton. (But not necessarily. Unified theories without proton decay exist. A scheme based on $[SU(3)]^3$ with permutation symmetry is an example.) These gauge bosons must therefore be extraordinarily heavy. (2) The value of the weak mixing angle $\sin^2 \theta$ can be computed, and the result agrees with experimental determinations.¹⁴ This is the only convincing quantitative indication that unification is correct. (3) An interplay between CP violation and baryon number violation can, during the very early history of the Universe, produce the observed baryon surplus.¹⁵⁻¹⁹ This result requires the existence of a rich structure of Higgs bosons,^{20,21} a fact which is confirmed by the observed pattern of fermion masses. For unified schemes based on either $SU(5)$ or $O(10)$, the Higgs bosons which couple to fermions must comprise at least two inequivalent representations. For $SU(5)$, at least a 5-plet and a 45-plet are needed. For $O(10)$, at least a 10-plet and a 126-plet are needed. In either case, the Higgs sector is then rich enough to provide the baryon surplus.

The superheavy unification mass may be estimated in two ways. Strong interactions are asymptotically free, so that the strong gauge coupling constant decreases with increasing energy. The fine-structure constant slowly increases. The point of convergence of the coupling constants dictates the unification mass. We obtain $\sim 10^{14}$ GeV assuming that we have correctly identified H as the entire phenomenological gauge group^{22,23}. The existence of baryon-number violating interactions must soon reveal itself.

An alternative estimate of the unification mass does not depend on the details of particle phenomenology. It follows from the present lower limit on the proton lifetime of 10^{29} yr (ref. 24). This assures us that the mass M of the baryon-number violating intermediary must be $\geq 10^{14}$ GeV. It also provides our upper limit on the strength of the fine-structure constant. We have noted that electromagnetism is not asymptotically free. The fine-structure constant depends on energy according to^{25,26}

$$\alpha^{-1}(0) = \alpha^{-1}(M^2) + \frac{2}{3\pi} \sum_f Q_f^2 \ln \left(\frac{M_w}{m_f} \right) + \frac{1}{18\pi} [32F - 63] \ln \left(\frac{M}{M_w} \right)$$

Where $M_w \approx 85$ GeV is the intermediate weak vector boson mass, Q_f and m_f are the charge and mass of the fermion f , and F is the number of families ($F \geq 3$). For this analysis to be sensible, $\alpha(M)$ is assumed to be < 1 . It follows that $\alpha(0)$ must be quite small. Since M is known to be $> 10^{14}$ GeV, one obtains the constraint, $\alpha^{-1}(0) \geq 25$. In any unified scheme in which the proton decays (but not too quickly), and for which perturbative estimates are reliable, the fine structure constant cannot be large. To a greater extent than was imagined, things must be as they are.

Received 12 June; accepted 17 August 1979.

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Direct observation of the structure of a metallic alloy glass

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Under favourable circumstances, high resolution axial bright-field transmission electron micrographs can provide information on the medium-range structure of oxide glasses and amorphous semiconductors¹. Specifically, the presence in an image of localised patterns of fringes, with the fringe spacing related by Bragg's law to the first diffraction peak, provides evidence of correlations in atomic positions over distances in the range 8-15 Å. 'Amorphous lattice fringes' with these characteristics have been studied in many amorphous solids but hitherto have not been reported in glassy metals, although several attempts have been described previously²⁻⁴. We present here what we believe to be the first high resolution axial, bright-field micrographs with lattice resolution of an amorphous metal, taken with the Cambridge University 600 kV high resolution electron microscope (HREM)⁵. The image features obtained indicate that it is now possible to observe directly the microstructure of amorphous metals, thereby emphasising the potential of high resolution microscopy as a tool for furthering the understanding of the structure of amorphous metallic glasses.

A bright-field electron micrograph provides a representation of the atomic density of the specimen projected onto a plane normal to the electron beam. The image contrast corresponding to a Fourier component of the specimen with period Λ is the product of the scattered amplitude, represented by the square root of the structure factor and the instrumental response function. The form of the latter, commonly known as the contrast transfer function, depends on the aberration constants of the objective lens and the electron wavelength, λ . It is controlled in practice by the strength of the objective lens, as characterised by a defocus parameter⁶ and modulated by envelope functions which represent the effects of non-monochromaticity of the incident electron beam and non-parallel illumination. Thus the contrast transfer function, $T(Q)$, is a damped oscillatory function of spatial frequency, $1/\Lambda$, with an amplitude which decreases rapidly for $\Lambda \leq 4$ Å for an accelerating voltage of 100 kV, as shown in Fig. 1a. However, by using a very strong objective lens, it is possible to extend the CTF to better than 2 Å although reversals in contrast make image interpretation difficult⁷.

For crystalline specimens, the structure factor, $S(Q)$ (where $Q = 2\pi/\Lambda$) consists of a series of intense peaks sharply localised in reciprocal space. Consequently, even though the magnitude of $T(Q)$ may be very low, it is often possible to arrange that

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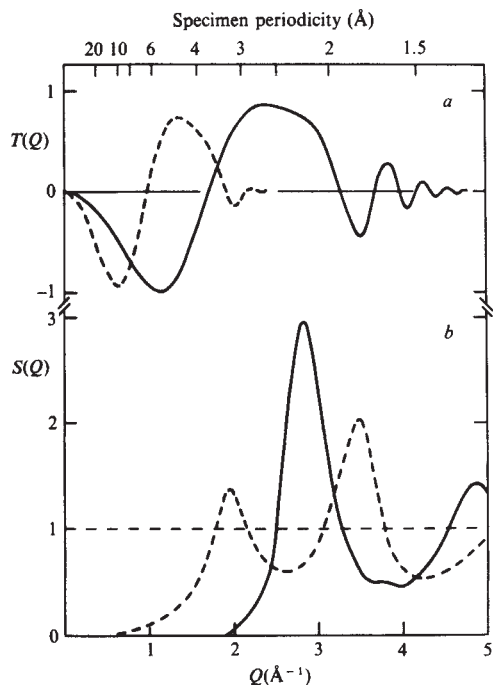


Fig. 1 *a*, Typical contrast transfer functions for electron microscopes with accelerating voltages of 100 kV (dashed line) and 500 kV (solid line). These functions are plotted for a generalised defocus value (see ref. 6) of $D = 3^{1/2}$ and with chromatic (C_c) and spherical (C_s) aberration coefficients, appropriate to typical 100 kV microscopes ($C_c = C_s = 1.8$ mm) and to the Cambridge HREM ($C_s = 3.27$ mm, $C_c = 3.33$ mm). *b*, Structure factors for α -Pd₄Si taken from neutron scattering data¹⁰ (solid line) and for α -Ge (X ray)¹¹ (dashed line).

$T(Q)$ is effectively constant over the width of the peak, and that the product $T(Q) \times S(Q)^{1/2}$ is large enough for lattice fringes of adequate contrast to be observed. The intensity of the first diffraction peak for any amorphous solid is much lower than that for a crystal, and the peak is characteristically diffuse—an 'amorphous halo'. As a direct result, faithful reproduction of any periodicities in amorphous solids generally presents more difficulties and, especially at high spatial frequencies, makes extreme demands on the performance of the microscope.

To observe 'lattice' fringes in an amorphous solid, it is apparently necessary to arrange that $T(Q)$ is maximised at Q_p , corresponding to the first peak of $S(Q)$, and that a broad peak, or lobe, of the contrast transfer function, encompasses a significant fraction of the first diffraction halo⁸. For materials such as amorphous silica, carbon, germanium and silicon, $Q_p = 1.5$ – $2.0 \text{ \AA}^{-1}$, these constraints can be met at 100 kV by arranging that the second or third lobe of $T(Q)$ shall coincide with Q_p . Observation of the image at 100 kV becomes almost impossible for amorphous metallic alloys with $Q_p \sim 2.8 \text{ \AA}^{-1}$ corresponding to $2.3 \text{ \AA}$ periodicities. At such high spatial frequencies, $T(Q)$ is effectively zero, unless the spherical aberration constant of the objective lens is very low, and normally oscillates several times (giving alternate positive and negative contrast) over the width of the diffraction peak.

The shorter electron wavelength corresponding to the higher accelerating voltage (up to 0.6 MV) of the Cambridge University HREM effectively extends $T(Q)$ to higher spatial frequencies by a factor approximately proportional to $(\lambda_{500}/\lambda_{100})^{1/2} \approx 1.6$ (ref. 9). For an accelerating voltage of 500 kV, $T(Q)$, in optimum conditions, now extends beyond $Q_p = 2.8 \text{ \AA}^{-1}$ (see Fig. 1*a*) so that the direct observation of any fringes present in amorphous metals becomes practicable. Moreover, as Fig. 1*b* shows, amorphous metals possess an important characteristic which militates in favour of clearer lattice images. Whereas the first peak of $S(Q)$ is almost invariably weak in amorphous semiconductors and oxide glasses, the

first peak in amorphous Pd₄Si is strong. Also, the absolute value of the scattered intensity—proportional to the square of a weighted sum of the atomic scattering factors—is typically more than five times larger than that for amorphous carbon. Consequently, the ratio of signal from an amorphous metal foil to noise from a carbon support film, or from a layer of contamination, may be acceptably high even for very thin specimens. This is of considerable importance since any ordered domains in amorphous solids would be expected to be smaller than 15–20 Å (on the evidence of both diffraction data and dark-field microscopy). Clearly, contrast is maximised for foils of thickness near 15 Å and decreases rapidly with foil thickness thereafter, due to overlap of domains which may not be Bragg-orientated and which thereby add to the background noise.

Thin foils of a palladium–silicon alloy were prepared by flash evaporation of a crystalline alloy from a tungsten spiral onto freshly cleaved rock salt at 20 °C at a pressure of around 2×10^{-5} torr. (Other foils have been prepared by evaporation onto liquid N₂-cooled substrates at a lower pressure and these give qualitatively similar image features.) Several films of different composition were prepared and specimens selected which were amorphous, as judged by electron diffraction and confirmed by dark-field microscopy which revealed no large speckle. The composition of the foil is not known with certainty—there seems to be some loss of silicon during the flash evaporation process, but since the specimen chosen lies at the high-silicon end of the range of amorphous specimens, it was judged to contain 20–25 at. % Si. Examination at 100 kV showed that the films had a droplet morphology with particles appearing to be supported on a very thin amorphous film (silicon

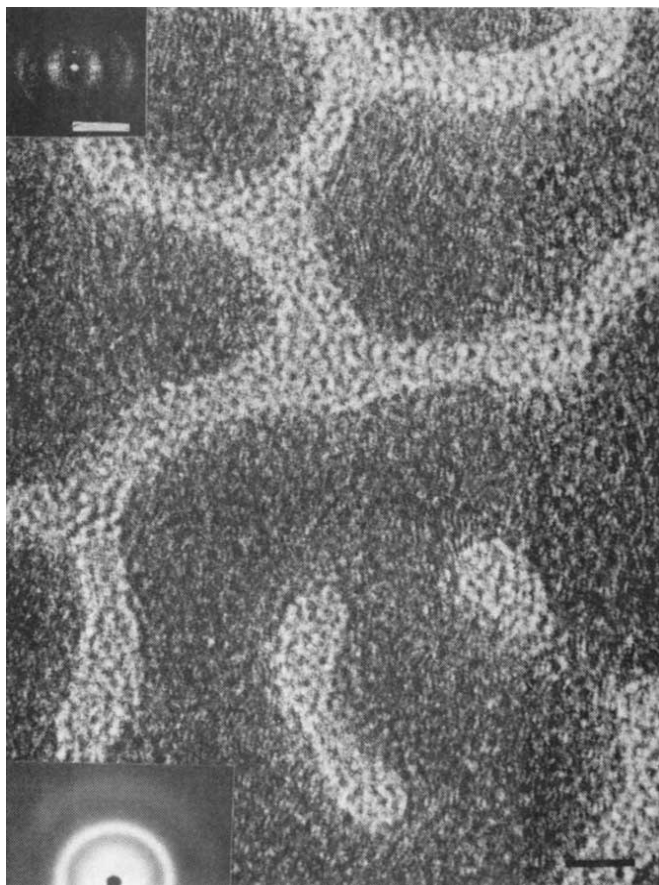


Fig. 2 High resolution image of an amorphous palladium–silicon alloy, recorded with an accelerating voltage of 500 kV. Fringe structure with a periodicity of about $2.3 \text{ \AA}$ is seen, especially in thin regions near the edge of the particles. Scale bar, $25 \text{ \AA}$. Insets show the image power spectrum (marker represents $(2.3 \text{ \AA})^{-1}$) obtained by computer processing (upper left) and the electron diffraction pattern (lower left).

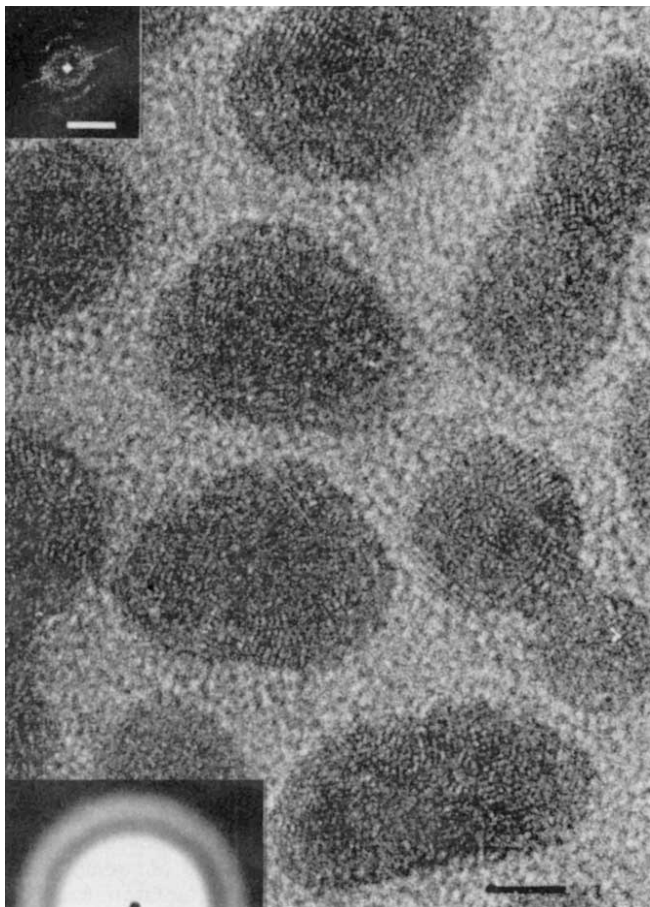


Fig. 3 High resolution image of amorphous palladium-silicon recorded in similar conditions to Fig. 2. The particles are smaller than those shown in Fig. 2 so fringe detail is more prominent.

dioxide). Some $4 \text{ \AA}$ structure was observed in this film but no high frequency detail was visible in the particles.

High resolution images recorded at an accelerating voltage of 500 kV show fringe-like features with a spacing of about $2.3 \text{ \AA}$ in the metal particles. In thicker specimens (Fig. 2) detail is most obvious, as expected, near the edge of the particles; at the centres, the structure gives the impression of randomness. Contrasting fringes are rarely seen, and then only over relatively small areas. The thickness profiles have not yet been determined, but assuming a circular cross-section, particles would be from 40 to $80 \text{ \AA}$ thick at their centres. It seems likely that the disappearance of fringe structure in the centre of particles is directly related to the overlap of Bragg-orientated regions with other, incorrectly-orientated domains.

Fringes are much more prominent in thin specimens, as evidenced by the image shown in Fig. 3. This specimen was evaporated at the same time as the thicker sample, with the thickness being varied by increasing the source-substrate distance. Although it is possible that this foil may have an essentially different structure from the thicker specimen it seems unlikely and the more pronounced structure is probably an effect of thickness. Only rarely do parallel fringe systems extend more than $20 \text{ \AA}$ in a given direction and the general impression gained from this micrograph is that each particle contains several fringe systems, some of which appear to be related by twin planes. In this sense, the particles seem to be similar to multiply-twinned particles in which lattice fringes are, however, more prominent, as identifiable multiply-twinned particles are often large and symmetrical (pentagonal or icosahedral) structures.

The fringe structures observed in Figs 2 and 3 show similarities to those seen in oxide glasses and amorphous semiconductors, in that parallel fringes are confined to local regions and the contrast is lower than that observed in single crystals of similar

size. There are, however, significant differences. Fringes are not only parallel but tend to be straight, in contrast to the curved fringes often seen in oxide glasses. Moreover, the area over which fringes are seen often exceeds $100 \text{ \AA}^2$. The electron diffraction patterns (insets in Figs 2 and 3) show the classical amorphous structure which does not appear to change with irradiation in the electron beam. Furthermore, diffraction patterns were recorded without any change of the illumination conditions from those used in first obtaining the micrographs (that is with a relatively weakly convergent beam), thus removing the possibility that the diffracted intensity from any electron-damaged region was swamped by the intensity scattered by the surrounding, unirradiated area. These figures display, perhaps more clearly than similar high resolution micrographs of amorphous semiconductors, that a locally ordered structure is not necessarily inconsistent with the impression of randomness beyond, say, $10 \text{ \AA}$ suggested by the diffraction information. The latter is an average over a specimen volume many orders of magnitude larger than that of the locally ordered domains observed in this film. A minimum conclusion appears to be that this specimen of amorphous Pd-Si possesses at least as much order as the oxide glasses and amorphous semiconductors. However, these micrographs are also inconsistent with any simple microcrystallite model.

Do the fringes necessarily imply localised order in the metallic glass? The possibility that statistically random fluctuations in a structureless medium could be imaged as fringes has been examined by several workers. Furthermore, periodic oscillations of the projected atomic density have been observed for certain orientations of (nominally) random models of amorphous metals¹². It is not possible to relate these 'pseudo-lattice' fringes to those observed experimentally except by careful computation of axial images. Calculations by Cochran¹³ and Krivanek¹⁴ suggest, however, that fringe-like regions with projected areas as large as those shown here are unlikely to arise by chance overlap of atoms in a truly random array.

Note that the results described here relate to a single example of an amorphous alloy, and that this specimen requires more adequate characterisation. Moreover, the electron diffraction technique is one of the least accurate for obtaining even semiquantitative scattering information. Examination of a more representative range of compositions is needed before the conclusion can be drawn that these specimens, with their relatively ordered medium-range structure, are typical of the whole class of amorphous metals. However, these results clearly reveal the potential of high resolution electron microscopy as a technique for examining the structure of rapidly quenched amorphous alloys. It should also be useful in studies of the puzzling low-temperature precrystallisation phenomena which have a drastic effect on the physical properties of many amorphous metals, and for following the rather complex series of crystalline transformation.

The Cambridge University 600 kV HREM has been constructed with major financial support from the SRC, to whom we are also grateful for individual support. P.H.G. acknowledges support from Pilkington Bros Ltd and from the SRC through a senior fellowship. We thank Drs V. E. Cosslett, W. C. Nixon and their colleagues, Dr A. Howie and Dr W. O. Saxton (who produced the power spectra shown in Figs 2 and 3) for helpful discussions.

Received 18 July; accepted 31 August 1979.

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Control of seawater composition

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We have previously discussed¹ the correlations between the seawater/rock partition function $K_Y(\text{sw})$ (mean concentration of Y in seawater/mean concentration of Y in crustal rock) and the electronegativity function $Q_{YO} = (x_Y - x_O)^2$ where x is the electronegativity of the subscripted element². The parameter Q_{YO} , which is a measure of the ionic interaction between the element Y and oxygen, was used to estimate the tendency of Y to become incorporated in the oxide-based mineral lattices which constitute most marine particulate matter. The nature of the correlations between $K_Y(\text{sw})$ and Q_{YO} (Fig. 2, ref. 1) led us to suggest that the partitioning of an element between seawater and crustal rock is controlled predominantly by its solid state chemistry with solution chemistry playing a secondary part. More recent estimates of the composition of mean world river water and mean crustal rock³ significantly improve the correlations described earlier and provide fresh data on the concentrations of the lanthanides in these reservoirs. Here we compare the chemistry of the lanthanides with that of alkali and alkaline earth metals with a similar electronegativity (the 'lithium group' Li, Mg, Ca, Sr, Table 1) to confirm that solid state chemistry plays a major part in controlling the composition of seawater.

Although they have similar electronegativities there are some striking differences between the behaviour of the lanthanides and the lithium group in the world oceans (Table 1). In the log $K_Y(\text{sw})$ versus Q_{YO} plot (Fig. 2, ref. 1) the partition coefficients for the lanthanides are among the lowest observed and lie at the end of the main sequence established by 60 elements. The partition coefficients for the lithium group lie on a separate correlation well above the main sequence and they have $K_Y(\text{sw})$ values some five orders of magnitude higher than lanthanides with a corresponding electronegativity (Table 1). Furthermore the lithium group elements have long oceanic residence times and can be described as 'enriched elements'⁶ as they are concentrated in the oceans relative to the world rivers (relative

oceanic enrichment $RE > 0$, Table 1). The lanthanides, however, are 'depleted elements'⁶ ($RE < 0$, Table 1) as they have short residence times in seawater and are effectively removed from solution on entering the world oceans. The scavenging of the lanthanides is very efficient as the concentrations of these elements in seawater are among the lowest observed for the stable elements. This is not primarily attributable to low crustal abundance since, for example, lanthanum, cerium and neodymium are more abundant than tungsten, cobalt and molybdenum in the Earth's crust¹⁰ although the latter elements are respectively two, three and five orders of magnitude more abundant in the oceans¹¹.

The reason for the effective removal of the lanthanides from the oceans is not to be found in their solution chemistry. Although the lanthanides in seawater are complexed a little more strongly than the lithium group, particularly by carbonate ions (Table 1), a substantial proportion of each element is present as the free ion so that both groups of elements behave as typical (a)-cations¹² with low to intermediate polarising power⁷ ($z^2/r < 11$, Table 1). The effective complexing of the lanthanides should favour higher concentrations of these elements in seawater than in river water. Solubility products do not suggest a suitable mechanism for the efficient removal of the lanthanides from seawater. Indeed, while the concentrations of some of the lithium group elements (such as Ca) are limited in seawater by the precipitation of insoluble salts, the lanthanides are several orders of magnitude below the concentration limits set by their insoluble salts (Table 2). For an explanation, therefore, we consider the differences in solid state chemistry between the two groups of elements. A key point here is the concept of 'capture' introduced by Goldschmidt¹⁴ to describe the situation in which the incorporation of a subordinate element into a mineral lattice in place of the dominant rock-forming elements results in a significant stabilisation of the surrounding crystal structure. Ion capture results when a site occupant is replaced by an ion with higher polarising power⁷ (z^2/r) and/or one which can accommodate a higher coordination number. The capture of lanthanide ions by calcitic minerals is common^{10,14} because the lanthanides have a higher polarising power than the alkaline earth cations of similar electronegativity (Table 1) and can accommodate higher coordination numbers¹⁵. Although Goldschmidt¹⁴ originally introduced the concept of capture in discussing the formation of igneous rocks it is possible that the same process will be apparent in marine diagenesis with the adsorption of the ion onto the mineral surface being followed by ion capture. This process will

Table 1 Comparison of the properties of lanthanide and Group IA and IIA cations

Cation type	Cation	x^*	r (Å) [†]	z^2/r	log K_Y (sw) [‡]	log $\bar{r}_Y$ (yr) [§]	RE	Seawater speciation [¶]					
								free	OH	F	Cl	SO ₄	CO ₃
Cerium earths	La	1.1	1.06	8.5	-5.69	3.31	-1.22	31	4	1	16	16	33
	Ce	1.05	1.03	8.7	-6.48	2.63	-1.90	22	4	1	11	21	41
	Eu	1.1	0.95	9.5	-5.63	3.53	-1.00	11	7	1	5	8	68
Yttrium earths	Gd	1.1	0.94	9.6	-5.52	3.47	-1.06	11	4	1	6	13	65
	Y	1.3	0.89	10.1	-5.95	—	—	16	14	4	7	4	55
	Lu	1.2	0.85	10.6	-4.90	3.83	-0.70	7	7	1	2	3	81
Group IIA	Mg	1.2	0.72	5.6	0.35	7.06	2.53	87	—	—	—	12	1#
	Ca	1.0	1.00	4.0	-0.59	5.98	1.45	89	—	—	—	10	1#
	Sr	1.0	1.16	3.5	-0.09	6.65	2.12	85	—	—	—	15	—
Group IA	Li	1.0	0.74	1.4	-0.92	5.71	1.18	99	—	—	—	1	—

* Ref. 4.

† Ref. 5.

‡ Recalculated from data given in ref. 3.

§ Mean oceanic residence time calculated from river input of dissolved material⁶. Residence times an order of magnitude lower are obtained if the particulate loads in river water and seawater are also taken into account¹⁰.

|| Relative oceanic enrichment, $RE = \log(\text{concentration in seawater}) - \log(\text{concentration in river water})$: $RE = \log \bar{r}_Y - 4.53$ (ref. 6).

¶ Whitfield *et al.*⁷ using data interpolated from critical compilations of stability constants^{8,9} for seawater at pH 8 and 35‰ salinity. Abundances quoted to nearest whole per cent.

Bicarbonate.

Table 2 Limitations set on the concentration of the lanthanides in seawater by the solubilities of their hydroxides, carbonates and phosphates

Element	Hydroxides		Carbonates		Phosphates		log [Ln] _{obs} #
	log K _S (OH)*	log [Ln] _{max} †	log K _S (CO ₃) ‡	log [Ln] _{max} §	log K _S (PO ₄)	log [Ln] _{max} ¶	
La	21.7	-2.3	-26.4	-6.3	-18.4	-8.7	-11.2
Ce	21.3	-2.7	—	—	—	—	-10.7
Eu	18.9	-5.1	—	—	—	—	-13.0
Gd	17.0	-7.0	-25.2	-5.7	-18.3	-8.6	-12.4
Y	18.9	-5.1	-23.6	-4.9	—	—	-11.6
Lu	15.9	-8.1	—	—	—	—	-13.2

* Solubility product for the reaction $\text{Ln}(\text{OH})_3(\text{s}) + 3\text{H}^+ \rightleftharpoons \text{Ln}^{3+} + 3\text{H}_2\text{O}$ at $I = 0.7\text{M}$ calculated using equations by Baes and Mesmer⁹.

† $\log [\text{Ln}]_{\text{max}} = \log K_{\text{S}}(\text{OH}) - 3 \text{pH}$ calculated for $\text{pH} 8$.

‡ Solubility product for the reaction $\text{Ln}_2(\text{CO}_3)_3(\text{s}) \rightleftharpoons 2\text{Ln}^{3+} + 3\text{CO}_3^{2-}$ at $I = 0.7\text{M}$ calculated from data by Smith and Martell⁸ assuming $g_{\text{CO}_3^{2-}} = 0.1$ and $g_{\text{Ln}^{3+}} = 0.01$, where g is the free ion activity coefficient.

§ $\log [\text{Ln}]_{\text{max}} = (\log K_{\text{S}}(\text{CO}_3) - 3 \log [\text{CO}_3]) / 2$ calculated for $\log [\text{CO}_3] = -4.58$ (ref. 7).

|| Solubility product for the reaction $\text{Ln PO}_4(\text{s}) \rightleftharpoons \text{Ln}^{3+} + \text{PO}_4^{3-}$ at $I = 0.7\text{M}$ calculated from data by Smith and Martell⁸ assuming $g_{\text{Ln}^{3+}} = g_{\text{PO}_4^{3-}} = 0.01$.

¶ $\log [\text{Ln}]_{\text{max}} = \log K_{\text{S}}(\text{PO}_4) - \log [\text{PO}_4]$ calculated for $\log [\text{PO}_4] = -9.7$ (refs 11, 13).

Seawater concentrations from Brewer¹¹, corrected for complexation of the lanthanide cations (Table 1).

be particularly effective in removing the lanthanides from solution where new minerals are produced in the oceans either by reverse weathering¹⁶ or by biological processes. There is, in fact, abundant evidence for the enrichment of the lanthanides in a range of authigenic minerals including glauconites, phosphorites, foraminiferal tests and manganese nodules^{10,17-20}. It is likely that the oceans are depleted in the lanthanide elements relative to the world's rivers because these effects only become apparent when the elements are held in a reservoir with a long retention time (several thousand years at least, Table 1). Evidence for the progressive incorporation of the lanthanides into phosphorites on ageing²¹ supports this hypothesis.

The suggestion that ion capture controls the chemistry of the lanthanides in seawater is supported by the differences in behaviour noted between the lanthanides themselves. In comparison with chondritic (non-ferrous meteoric) minerals the lighter lanthanides (the 'cerium earths' La-Eu) are enriched in preference to the heavier lanthanides (the 'yttrium earths', Gd-Lu and Y) in most hydrogenous minerals¹⁰. This preferential enrichment probably arises because the effect of the increasing polarising power of the cation (z^2/r , Table 1) on going from La to Lu is outweighed by the higher coordination number which the larger, lighter cations are able to accommodate in oxide lattices¹⁵, thus favouring their capture. As a result of their preferential enrichment in mineral structures the 'cerium earths' have significantly shorter residence times (t_V) in the oceans and lower partition coefficients ($K_V(\text{sw})$) than the 'yttrium earths' (Table 1). The plot of $K_V(\text{sw})$ against $Q_{Y\text{O}}$ (Fig. 2, ref. 1) shows that these differences are adequately represented by the electronegativity function, $Q_{Y\text{O}}$.

Thus, the chemistry of the lanthanides supports our original deduction¹, based on the distribution of the elements along the $K_V(\text{sw})$ versus $Q_{Y\text{O}}$ plot, that the overall composition of seawater is controlled by the solid-state chemistry of the elements. The comparison between the lanthanides and the lithium group also clarifies the mechanism by which solid-state control is achieved in the case of the electropositive elements.

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Iron-nickel superstructure in metal particles of chondrites

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Ordered solid solutions (superstructures or superlattices), in which atoms of one kind segregate into a particular set of lattice positions, are usually obtained by slow cooling at the critical ordering temperature. However, the equiatomic Fe-Ni ordered phase (superstructure L10) has been obtained only by radiation-induced ordering¹, since the diffusion rates of the metallic atoms are very slow at the ordering temperature $T_c = 320^\circ\text{C}$ (ref. 2). The Fe-Ni ordered phase has recently been detected by Mössbauer spectroscopy and X-ray diffraction in the taenite of the iron meteorites Cape York (III A), Toluca (I)³ and reported as a major constituent of the Ni rich ataxite (35% Ni) Santa Catharina⁴. We report here Mössbauer spectra, scanning electron microscopy and X-ray measurements, which show that the metal particles of the chondrites Saint-Séverin (LL6), Lake Labyrinth (LL6) and Parambú (polymitic LL breccia) contain large proportions of this Fe-Ni superstructure and that the phase is present in different amounts in the chondrites L'Aigle (L6), Peetz (L6), Shaw (L6), Björbole (L4) and Allegan (H5).

The identification of the L10 superstructure in Fe-Ni alloys by Mössbauer spectroscopy is based on the fact that the ordered phase exhibits an asymmetric 6-line spectrum due to a quadrupole splitting arising from the non-cubic environment of the Fe atoms in this structure. Detection by X-ray diffraction is more difficult due to the very weak intensity of the superstructure lines⁵.

Received 16 July; accepted 31 August 1979.

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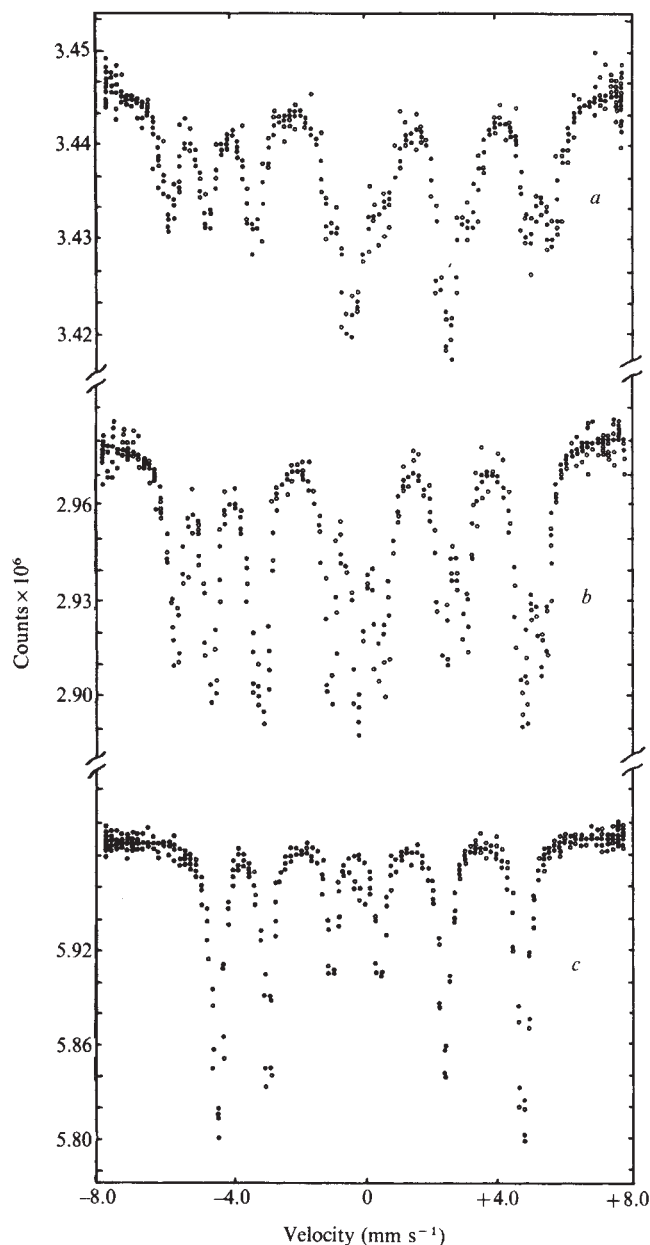


Fig. 1 Mössbauer spectra of St Séverin metal fraction at room temperature recorded with a sinusoidal drive and HP multichannel analyser (1,024 channels). The data were fitted with a 370/145 IBM computer. *a*, Untreated metal fraction magnetically separated and sieved to 40–80 μm . *b*, Metal fraction (80–150 μm) after HF treatment. *c*, Ordered phase in pure form extracted from metal fraction. The quadrupole effect is seen in the asymmetric position of the outer lines. From spectrum *c* one identifies the lines corresponding to the ordered phase in the upper spectra *b* and *a*.

Chondrites exhibit complex Mössbauer spectra⁶ which arise from the superposition of: (1) quadrupole interaction from the paramagnetic iron silicates which yields two pairs of doublets. (2) Magnetic hyperfine interaction from the α -phase (kamacite) or the α_2 -phase (martensite), γ -phase (taenite) and troilite, which yields three six-line spectra resulting in a total of 18 partially overlapping lines. (3) A single central line arising from paramagnetic γ -phase (taenite).

With magnetically enriched samples relatively large amounts of silicates and troilite still remain attached to the metallic phase. Only in a favourable case such as St Séverin, which contains a large proportion of the ordered phase, was it possible to detect the superstructure by Mössbauer spectroscopy without further purification of the sample (Fig. 1*a*).

We found that a separation of the metals from the silicates (up to about ~95%) and the troilite can be achieved by boiling the metallic fraction in concentrated HF (40%) for a few minutes and submitting the residue to a magnetic separation. This treatment does not affect the metallic phase, as we observed in experiments with several Ni-rich Fe-Ni alloys. Moreover the Ni/Fe ratios estimated from the areas corresponding to the several metallic phases observed in the Mössbauer spectra (Fig. 1*b*) are in reasonable agreement with those reported by chemical analysis.

Table 1 lists the hyperfine parameters derived from the Mössbauer spectra of the chondrite metals in which the ordered phase has been observed, as well as the proportions of the Fe-Ni alloy phases in these meteorites.

The relatively large Ni/Fe ratio found in the metallic phase of the LL chondrites favours the formation of the L10 superstructure which also seems to be common in the L chondrites, although in smaller proportions. The presence of the ordered phase in the H chondrite Allegan was confirmed by a Debye-Scherrer diagram (filtered $\text{CoK}\alpha$) of an untreated metal particle presenting a cloudy taenite border which showed the α -phase, the γ -phase and (100), (110) and (210) superstructure lines (J. Laugier, personal communication).

Within the sensitivity of the Mössbauer technique (up to a few per cent) no evidence for the ordered phase was found in the

Table 1 Hyperfine parameters of the chondrite metal fraction

Chondrites	α -phase				Ordered γ -phase				γ -phase (paramagnetic)			
	IS (mm s^{-1})	H (kOe)	W (mm s^{-1})	A (%)	IS (mm s^{-1})	ΔE_Q (mm s^{-1})	H (kOe)	W (mm s^{-1})	A (%)	IS (mm s^{-1})	W (mm s^{-1})	A (%)
Metal fraction												
St Séverin	-0.09	343	0.43	39.9	-0.07 ± 0.05	$+0.20 \pm 0.01$	289	0.43	50.8	-0.16	0.38	9.5
Lake Labyrinth	-0.11	322	0.76	50.7	-0.03 ± 0.03	$+0.13 \pm 0.05$	288	0.51	36.6	-0.16	0.45	9.8
Parambú	-0.07	340	0.50	37.8	-0.08 ± 0.02	$+0.16 \pm 0.02$	294	0.50	42.7	-0.12	0.46	9.8
L'Aigle	-0.10	338	0.45	74.1	-0.14 ± 0.03	$+0.16 \pm 0.05$	302	0.45	16.0	-0.13	0.46	9.8
Björbole	-0.09	339	0.44	85.2	-0.04 ± 0.03	$+0.24 \pm 0.03$	298	0.44	10.9	-0.14	0.33	3.9
Allegan	-0.09	335	0.38	64.5	-0.02 ± 0.04	$+0.22 \pm 0.03$	287	0.38	17.5	-0.06	0.43	10.7
Peetz	-0.10	343	0.57	86.3	-0.19 ± 0.07	~0	300	0.57	7.8	-0.10	0.78	5.9
Shaw	-0.10	341	0.48	62.2	-0.09 ± 0.03	~0	298	0.48	17.4	-0.03	0.67	9.5
Enriched ordered phase												
Parambú	—	—	—	—	-0.07 ± 0.007	$+0.12 \pm 0.01$	288	0.49	70.7	—	—	—
St Séverin	—	—	—	—	-0.07 ± 0.002	$+0.21 \pm 0.01$	287	0.35	100.0	—	—	—

IS, The isomer shift relative to a 25 mC Co^{57} in Rh (error ± 0.01 in α -phase and ± 0.04 mm s^{-1} in paramagnetic γ -phase); ΔE_Q , quadrupole splitting; H, magnetic field (± 5 kOe); W, linewidth (± 0.03 mm s^{-1} in the α and ordered phase, and ± 0.3 mm s^{-1} in the paramagnetic γ phase); A, area ($\pm 5\%$), <10% iron silicates are present in Shaw, Allegan, Parambú, and <5% in the others. The ordered phase in Parambú was enriched up to 70% and up to ~100% in St Séverin.

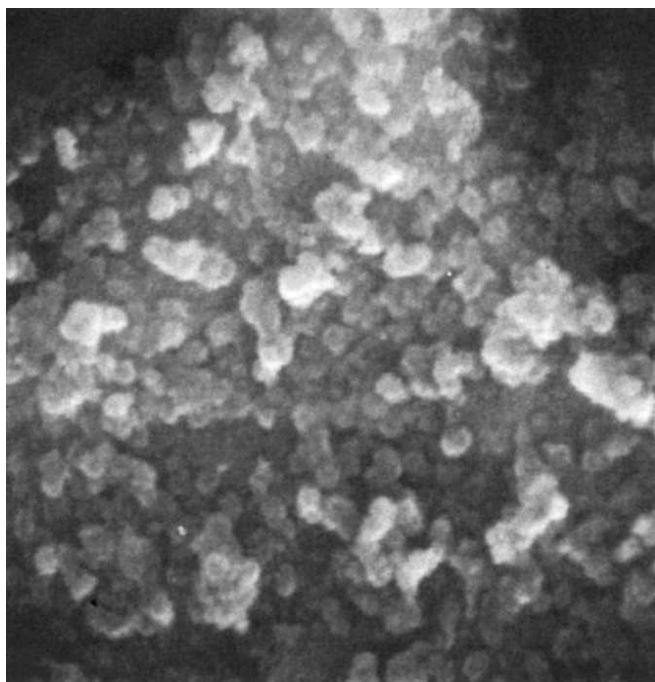


Fig. 2 Scanning electron micrograph ($\times 20,000$) of the Fe-Ni ordered phase from St Séverin.

metal phases of the H chondrites Nadiabondi, Menow, Ipiranga, Marília, São José do Rio Preto, Avanhandava and Estacado. The X ray of a Nadiabondi metal grain, however, exhibited a faint (100) superstructure line.

We were able to isolate the Fe-Ni ordered alloy as a single phase constituent. In a Cu^{2+} solution both Fe(0) and Ni(0) metals are dissolved⁷ but the rate of dissolution is slower for the ordered phase. A fraction in which this phase is enriched can be obtained by treating the metallic fraction (after HF) for several minutes in a $\text{CuCl}_2/2\text{KCl}$ solution. Cu(0) is next eliminated from the metal residue with concentrated ammonia. A sample extracted from St Séverin exhibits a Mössbauer spectrum which corresponds to an essentially pure ordered phase (Fig. 1c). The composition of the alloy in this sample was found to be Fe(60%) and Ni(40%), in the domain of stability of the Llo superstructure².

Scanning electron microscopy showed an assembly of small structures (Fig. 2), similar to those reported by Scott in crystallographic studies of cloudy taenite⁸, with sizes between 1,500 and 3,000 Å and quite homogeneous metal composition.

The parameter $a_0 = 3.579 \pm 0.001$ Å obtained by X-ray diffraction of this sample is that reported for the ordered phase^{9,10} and is smaller than that corresponding to the disordered alloy $a_0 = 3.596 \pm 0.001$ Å. With a $\text{CoK}\alpha$ radiation in a 24-h exposure the (100), (110), (210), (211) and (221) weak superstructure lines were detected in the Debye-Scherrer diagram¹¹.

The value of the quadrupole splitting of the ordered phase extracted from Parambú ($+0.12 \pm 0.01$ mm s^{-1}) is significantly smaller than that observed with St Séverin ($+0.21 \pm 0.01$ mm s^{-1}). Parambú is a polymitic breccia with evidence of a shock event¹² whereas St Séverin presents little evidence of shock. This suggests that the shock event, either from pressure effects¹³ or by impact heating, tends to disorder the Fe-Ni superstructure ($T_c = 320^\circ\text{C}$), as indicated by the decrease in the value of the quadrupole splitting¹⁴. This would explain the low value ($\Delta E_Q = +0.13 \pm 0.05$ mm s^{-1}) observed with Lake Labyrinth, which according to Taylor and Heymann¹⁵ is a heavily shocked chondrite (class C(H)).

The cases of Peetz and Shaw are particularly interesting since their Mössbauer spectra corresponds to the α (or α_2) phase, as in the other chondrites, and a ferromagnetic γ phase with same

hyperfine parameters of the superstructure but largely disordered ($\Delta E_Q \sim 0$). The metallic phases in Peetz indicated evidence of shock, but only moderate reheating¹⁵. The relatively large negative value of the ^{57}Fe isomer shift observed with Peetz is compatible with a pressure effect above 200 kbar¹⁶.

Recent studies indicate that Shaw is a complex meteorite which experienced an unusual event, superimposed on a normal slow cooling history, probably connected with an intense shock¹⁷⁻²⁰. The destruction of the superstructure in Shaw could arise by impact reheating of the meteorite to temperatures above 320°C and rapid cooling to lower temperatures which prevented the subsequent ordering of the Fe-Ni alloy.

We thank J. M. Knudsen for his continued interest, J. Laugier, K. Imakuma and H. A. Abdel-Rehim for X ray measurements, and C. Vilbert for scanning electron microscope measurements. Metal fraction of chondrites were supplied by P. Pellas and Brazilian chondrite samples by C. B. Gomes.

Received 5 June; accepted 21st August 1979.

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Ionospheric-magnetospheric contributions to storm-time magnetic field changes near dip equator

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Experimental identification of the electric current systems in the ionosphere and magnetosphere during magnetic storms is a fundamental problem. We report here a new approach to resolving the relative contributions of ionospheric and magnetospheric currents to the storm-time geomagnetic field variations at equatorial latitudes. In this approach, the VHF backscatter radar-measured electric field changes in the equatorial electrojet and the simultaneous surface magnetic field changes are analysed to determine quantitatively the ionospheric contribution ΔF_I to the storm-time geomagnetic field perturbation ΔF_D . The magnetospheric contribution ΔF_M is then determined from:

$$\Delta F_D = \Delta F_I + \Delta F_M \quad (1)$$

This method has yielded extremely interesting results for the magnetic storm of 15 February 1978. It has a great potential for providing valuable new insights into the nature and time evolution of the complex storm-time current systems in the ionosphere and magnetosphere.

A coherent VHF backscatter radar and a proton precession magnetometer (PPM) were used for storm-time observations at Thumba near Trivandrum (dip 56° S; lat, 8.5° N; long, 77° E). The two experimental systems are described elsewhere^{1,2}. Later improvements have enabled us to record the radar digital data on a magnetic tape and process it on an IBM 360/44 computer. The data recorded at 1-min intervals are used here. As the dip angle is very small (56° S) the approximation $\Delta H \approx \Delta F$ is used, where ΔH is the perturbation in the horizontal component of the magnetic field. All interpretations given here are based on this approximation. For the radar geometry at Thumba, the mean Doppler frequency $\bar{f}_D$ is negative when the drift of the ionisation irregularities (and electrons) is westward under the action of an eastward electric field. From the observed $\bar{f}_D$ fluctuations, the fluctuations of the electron drift velocity (which are directly proportional to electric field fluctuations) can be determined from standard theoretical relationships³.

Figure 1 shows the observed variations with time of the geomagnetic field perturbation ΔF , the backscatter signal strength V and the mean Doppler frequency $\bar{f}_D$ of the radar signals on 15 February 1978. A sudden commencement storm started at 03.17 IST (82.5° EMT). The most remarkable features in Fig. 1 are: (1) a delay of about 1.5 h in the first appearance of the radar signal (at 0905 h) indicates that the normal eastward electric field is suppressed between 0730 and 0900 h; (2) the large in-step fluctuations of ΔF , $\bar{f}_D$ and V between 0905 and 1430 h of the storm initial phase; (3) a large main phase decrease of ΔF to negative values between 1530 and 1630 h, even while $\bar{f}_D$ is increasing shows that a magnetospheric current is responsible for the large negative value of ΔF ; (4) a repeated disappearance of the radar signal for durations of 20–30 min, indicating the reversals of normal daytime eastward electric field in the electrojet; (5) the disappearance of large amplitude fluctuations at about 1800 h after the storm recovery phase has set in. Other interesting details of the observations shown in Fig. 1 will be

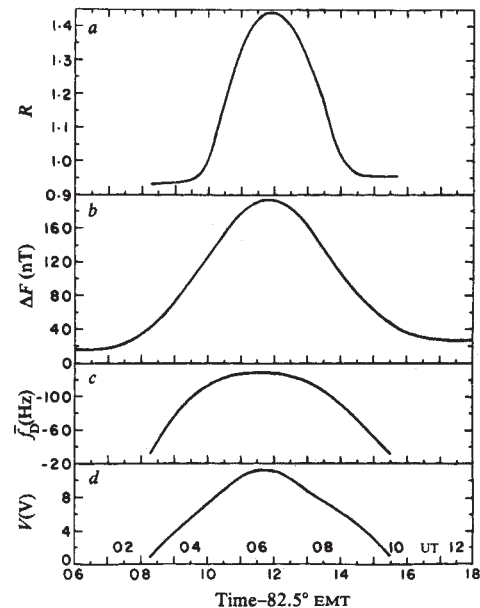


Fig. 2 The time variations of ΔF , $\bar{f}_D$ and V on 11 February 1978 (a quiet day with $A_p = 5$). $R = \Delta F/\bar{f}_D$ is also shown (a).

discussed later. The time variations of ΔF , $\bar{f}_D$ and V on 16 February 1978 were also recorded during the recovery phase of the storm.

The interesting and informative experimental observations shown in Fig. 1, along with similar observations on a quiet day, have been used to quantify the ionospheric and magnetospheric contributions to the storm-time surface magnetic field perturbations at Thumba. Figure 2 shows the time variations of ΔF , $\bar{f}_D$ and V on 11 February 1978 which is a quiet day with $A_p = 5$. In Fig. 2a, the ratio $\Delta F/\bar{f}_D$ is shown as R ,

$$R = \Delta F/\bar{f}_D \quad (\text{nT})(\text{Hz})^{-1} \quad (2)$$

Using the suffixes Q and D respectively for quiet and disturbed day values, the approximation $\Delta F_Q = \Delta F_I$ has been used instead of $(\Delta F_I)_Q = (\Delta F - D_{st})_Q$. A later check on 11 February D_{st} values shows that R value may be in error by 10–20% due to the non-subtraction of D_{st} values. Note that

$$\Delta F_I = k_1 \Sigma_3 E_y \quad \text{and} \quad \bar{f}_D = k_2 \bar{\mu} E_y \quad (3)$$

Here, $\bar{\mu}$ is a mobility parameter involving the collision and gyro frequencies of ions and electrons; k_1 and k_2 are constants of proportionality which are independent of the medium; E_y is the east-west electric field which varies very little with height if it is a global scale field and is assumed to be height-independent; Σ_3 is the height-integrated Cowling conductivity; and the bar denotes a weighted average over that height region which contributes to the backscatter signal. From equations (2) and (3), we can write

$$R = (k_1/k_2) \Sigma_3 / \bar{\mu} \quad (4)$$

Thus, the variation of R should represent the variation of Σ_3 , since k_1 and k_2 are constants and $\bar{\mu}$ is likely to have negligible variation with time of the day compared with that of Σ_3 .

If it is assumed that at any given local time the change in Σ_3 from a quiet day to a disturbed day is much smaller than that in the electrojet electric field, then ΔF_I on a disturbed day can be deduced from

$$\Delta F_I = R_Q (\bar{f}_D)_D \quad (5)$$

Then the magnetospheric contribution ΔF_M is determined from equation (1). Obviously, the errors in ΔF_I and ΔF_M deduced in this way are proportional to the difference $(R_D - R_Q)$. The fractional change in R from quiet to disturbed day is not likely to exceed 15–20% most of the time while the ΔF_I and ΔF_M changes from quiet to storm day are in the range of 50–200%. Pending a

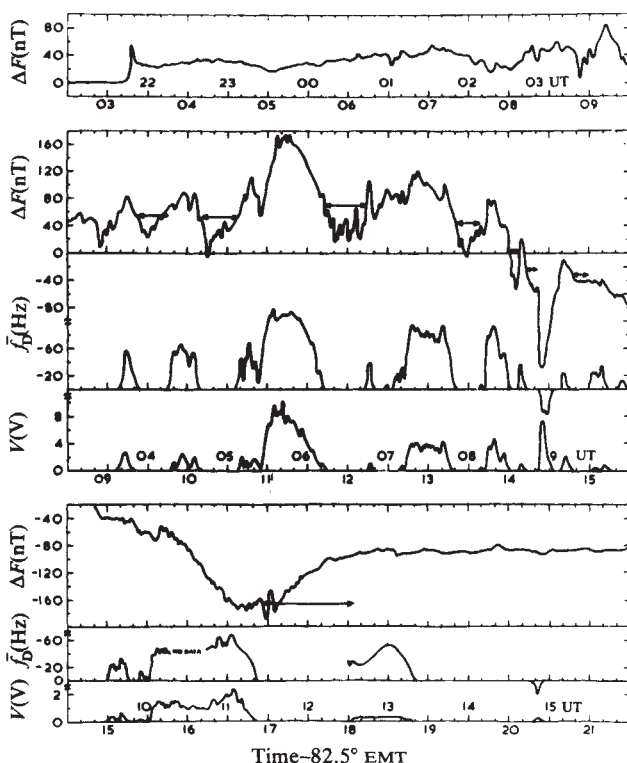


Fig. 1 The time variations of the surface magnetic field strength ΔF , the mean Doppler frequency $\bar{f}_D$ and the strength V , of the VHF backscatter radar signals on 15 February 1978. The horizontal arrows ($\leftrightarrow$) mark the periods during which the radar signals disappeared. $A_p = 48$. Time is 82.5° EMT.

further quantitative study of the $(R_D - R_Q)$ values, we assume that R_Q values can be used on a disturbed day to determine storm time ΔF_I and ΔF_M . Ideally, we need at least 4 or 5 quiet days' observations to arrive at the quiet day mean value of R for a given month. The induced Earth current effects are not separated out in the present analysis.

The ionospheric and magnetospheric contributions to the surface magnetic field perturbations during the 15 February storm have been estimated using the method outlined above. The results are shown in Fig. 3, where the chain curve represents the quiet day variation of ΔF and the dotted portions of the ΔF_M curve correspond to the times of disappearance of backscatter signals. If the eastward current and hence ΔF_I is assumed to be zero at such times, then $\Delta F_M = \Delta F_D$ at such times and this is the lower limit to ΔF_M . On the other hand, the broken portions of the ΔF_I and ΔF_M curves correspond to those times at which backscatter signals on the disturbed day are available but not on the quiet day, and hence an extrapolated value of R has been used. R is assumed to have its asymptotic value of 0.96 at such times.

The ΔF_I behaviour between 0900 and 1500 h indicates that the electrojet electric field and current are fluctuating violently during this time, with the average levels being rather low compared with quiet day values. The time variation of ΔF_I between 1500 and 1900 h shows that there is an enhancement of the eastward electric field and current values over the quiet day (11 February) levels in this time sector.

Consider the behaviour of ΔF_M . The circles in Fig. 3 represent the smoothed values of ΔF_M obtained through a 60-min running average of 1-min values. Between 0900 and 1400 h, ΔF_M is essentially positive and is subject to strong oscillations of 4–6 min period and to longer period (20–40 min) perturbations like ΔF_I . The 4–6 min oscillations are apparently hydromagnetic waves originating in the magnetosphere and propagating to the equatorial ionosphere⁴. In fact, detailed examination has shown that the short period oscillations as well as the longer period bay type perturbations in ΔF_I and ΔF_M are in antiphase with each other. Such simultaneous phase-coherent perturbations in ΔF_I and ΔF_M indicate that the magnetosphere, auroral ionosphere and equatorial ionosphere together respond as a single electrodynamical system to storm-time disturbance forces; and the

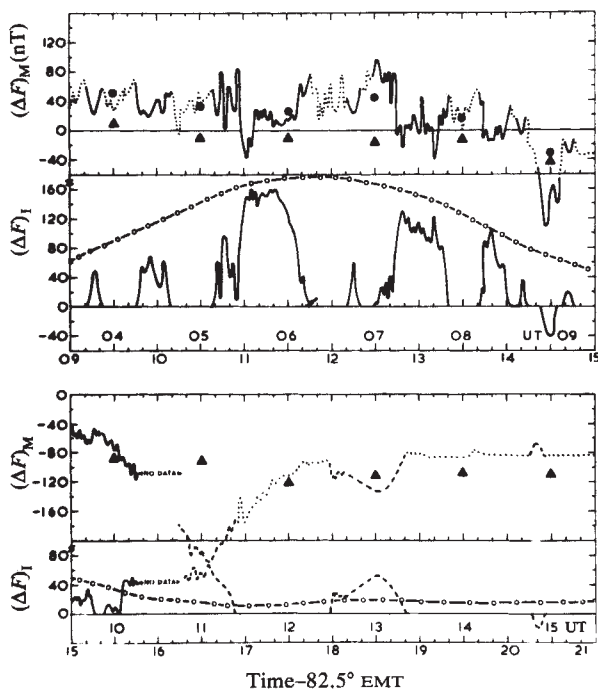


Fig. 3 The time variations of ΔF_I and ΔF_M on 15 February 1978. Solid line, ΔF_D ; $\circ$ — $\circ$, ΔF_Q ; $\blacktriangle$, D_{st} . $A_p = 48$.

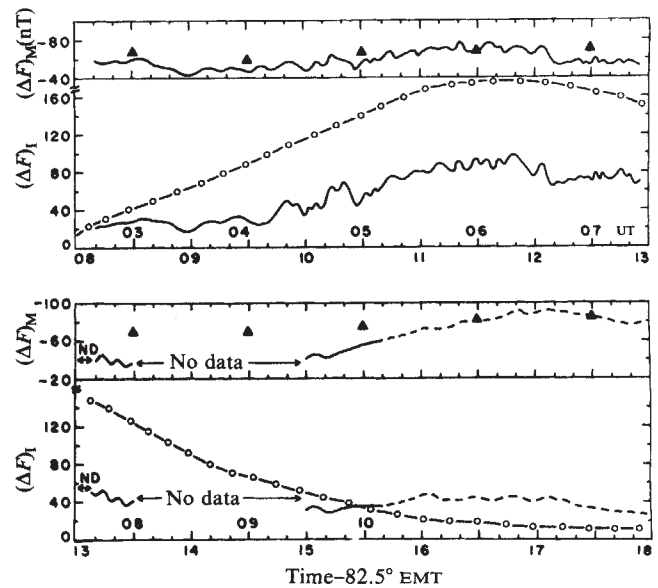


Fig. 4 The time variations of ΔF_I and ΔF_M on 16 February 1978. Solid line, ΔF_D ; $\circ$ — $\circ$, ΔF_Q ; $\blacktriangle$, D_{st} . $A_p = 11$.

interactions and feedback effects within this system cannot be ignored in any study of the physical processes. This and other features, when studied in detail, may provide important clues to the nature of magnetosphere-ionosphere responses and interactions during storms and substorms.

Moreover, the agreement between the D_{st} values and ΔF_M values is not good between 0900 and 1430 h. Apparently, the D_{st} analysis cannot correctly bring out the magnetospheric contribution to ΔF during this initial phase which is dominated by short-period oscillations and irregular perturbations.

The magnetospheric contribution ΔF_M begins to attain large negative values rather rapidly from 1520 h onwards. Significantly, the short-period oscillations and bay-type disturbances come to an abrupt stop during this phase of the storm. However, the large rapid decrease of ΔF_M and its recovery between 1600 and 1730 h are not shown up by the D_{st} analysis. But, the D_{st} values and ΔF_M values show a remarkable agreement after 1730 h. Obviously, during the late afternoon-evening sector, the surface magnetic field perturbation is caused predominantly by magnetospheric currents; and ΔF_M continues to be large throughout the night of 15 February.

Similar analysis has been done for 16 February, and the main results (shown in Fig. 4) for the recovery phase are: (1) During the recovery phase of the storm ΔF_I is still well below the quiet day value until 1400–1500 h IST; but ΔF_I clearly exceeds (by a factor of 2 or 3) the quiet day value between 1600 and 1800 h. These features indicate that a disturbance associated westward electric field persists in the equatorial electrojet during the recovery phase in the morning–noontime sector, followed by an enhanced eastward electric field in the late afternoon–evening sector. (2) On 16 February there is an excellent agreement in general between D_{st} and ΔF_M values (which are high), implying that once the symmetric ring current is well developed as a dominant feature of the magnetosphere, the procedure used for deducing the D_{st} values is able to reproduce its behaviour quite well.

The present method opens up new possibilities in quantitative studies on the equatorial electrojet current changes during different phases of magnetic storms. However, the quantitative accuracy of the present method can be improved only through further refinements in the methods of radar measurement and the data analysis. For example, the variation of R in Fig. 2 has a gross resemblance to the expected variation of Σ_2 but it shows important deviations as well. The causes of the deviations are

being investigated. Similarly, we are aware⁵ that local winds with vertical shear can modify and distort the height structure of the electrojet, but the wind-generated electric fields are usually much weaker than the large storm-time electric field changes. On the other hand, the excellent agreement between ΔF_M and D_M values over long periods of storm-time (particularly after the development of the ring current) cannot be fortuitous, and this lends support to the assumption that change in the R value from quiet to storm day is small compared to the changes in E_y . In any case the estimated errors in ΔF_I and ΔF_M can be as large as 25% in the first attempt, although we believe that the errors are much less for a considerable period of the storm-time.

Received 30 April; accepted 20 July 1979.

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Do sponges help hold coral reefs together?

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The growth and form of coral reefs is the result of a complex balance between rates of carbonate accretion and carbonate loss. Reef organisms have traditionally been classified, with respect to their role in this balance, as primary frame-builders, frame-cementers, biological eroders or sediment producers^{1,2}. Scleractinian corals are the primary frame-building organisms in most modern reef environments. The growth of many such corals generates large volumes of unoccupied cryptic space. Successful reef construction occurs if this space is infilled with sediment, this sediment is cemented by frame-cementing organisms, and the resulting complex is subsequently lithified^{3–5}. As this lithification process may be slow, frame material is highly susceptible to becoming separated from the reef framework due to the action of physical disturbance (such as wave shock) before permanent consolidation^{6–10}. We present here experimental evidence that demosponges (which may be second only to scleractinian corals in space occupancy on many Caribbean reefs) play an important role as interim binders of unconsolidated frame material, a process which is expected to increase rates of carbonate accretion.

Although sponges may bind carbonate to the reef frame in several reef zones (for example, interstices of ramose corals and carbonate rubble), our investigations have focused on Caribbean deep fore-reef environments. Deep fore-reef zones are characterised by steeply sloping sand plains dotted with small patch reefs or larger reef pinnacles and are bounded to the seaward by vertical rock walls which drop to considerable depths. Scleractinian corals growing in this zone generally exhibit a foliaceous growth morphology. Such forms are especially susceptible to disengagement from the reef since their attachment zones are small; their undersurfaces are largely devoid of live coral tissue, leaving them open to recruitment of boring organisms; and they extend off the reef rather than resting on it, making them unstable once their attachment zone is weakened¹¹. Foliaceous corals have become separated from the reef frame by the forces exerted by a feeding parrotfish, by the actions of storm-induced wave shock^{6–10}, by the fins of a careless scuba diver, or simply due to collapse under their own weight¹¹. Once separated from the reef, corals cannot reattach and are rarely consolidated.

Non-burrowing sponges characteristic of deep fore-reef environments are of two general types: those which inhabit the holes and crevices in the reef frame and those which inhabit exposed reef substrata. Cryptic sponges (such as *Agelas clathrodes* (Schmidt)) commonly have zones of attachment which include both the undersurface of foliaceous corals and the reef frame itself. The porosity of the reef frame may cause many of these sponges to extend from one attachment spot on the undersurface of a coral, through the reef frame to an attachment zone on another coral.

Sponges, including those which inhabit open reef substrata, have been observed to attach to one another without harm to either side^{12,13}. Experiments performed on the barrier reef of Belize show that several such sponges may also kill small patches of coral tissue on contact and bind to the exposed coral skeleton. Growing tips of at least five individuals of each of 12 sponge species were severed with a diving knife and attached to living agariciid corals with cotton twine. As controls, pieces of commercial bath sponge were attached to living corals. The contacts were examined after one week for evidence of coral tissue death and sponge–coral binding. Of the 12 species, 10 killed a small patch of coral tissue and bound to the skeleton (Table 1). The two remaining species killed small patches of coral. The bath sponge had no observable effect. The morphological flexibility of sponges, their ability to attach to other living sponges and corals, and their ability to attach to clean substrata with any part of their colony results in the formation of anastomosing networks of several sponge species which have many attachment points on different coral colonies and the reef frame.

Corals, although far more constrained in morphological flexibility by their rigid skeletons, can also modify their forms in the presence of certain sponges. We have observed colonies of *Agaricia agaricities* (Linn.), *Agaricia lamarcki* (E & H), and *Helioserius cucullata* (E & S) which have encircled the tissue and even attachment zones of nearby sponges with skeletal material. These observations may simply reflect a passive response of the coral to any obstruction within the path of growth.

Table 1 Sponge–coral binding experiment

Sponge species	Total no. of individuals tested	Total no. killing tissue	Total no. binding to coral
<i>Agelas clathrodes</i> (Schmidt)	5	5	0
<i>Agelas conifera</i> (Schmidt)	5	5	5
<i>Agelas scepstrum</i> (Lam.)	5	5	5
<i>Aplysina cauliformis</i> (Carter)	5	5	5
<i>Callyspongia</i> sp.	7	7	7
<i>Ectyoplasia ferox</i> (D & M)	10	8	8
<i>Haliclona rubens</i> (Palles)	30	25	15
<i>Iotrochota birotulata</i> (Higgin)	5	5	5
<i>Niphates erecta</i> D & M	10	5	0
<i>Smenospongia aurea</i> (Hyatt)	5	3	3
<i>Thalysias juniperina</i> (Lam.)	5	2	2
Unidentified	7	7	5
Control	5	0	0

Experiments were performed while operating from the RV Alpha Helix. Two locations, one each in the lee of Glovers and Lighthouse Atolls at depths of 20–30 m, were studied. The Lighthouse Atoll site is 1 mile south of Long Cay and the Glovers site on the reef pinnacle, Movie Mountain, located on the southwestern extreme of the atoll. The experiment does assist with the understanding of the mechanism (for example, allelopathy and abrasion) by which sponges kill coral tissue. As these results represent the number of individuals binding to corals in only 1 week, they probably underestimate the actual capacity of these sponges to bind to corals.

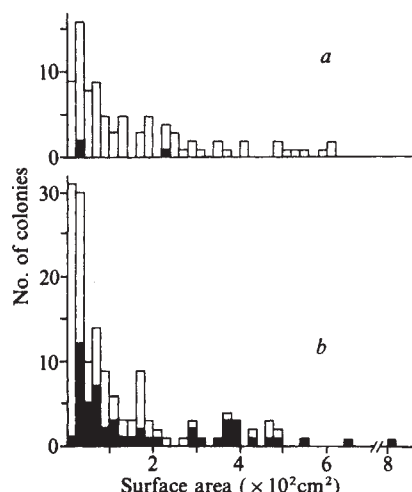


Fig. 1 Size against frequency distribution of foliaceous corals originally extant on *a*, unmanipulated reefs and on *b*, manipulated reefs. Shaded histograms represent those corals which became separated from the reefs. Patch reefs selected were comparable in size and sponge-coral faunal development. Specific data on patch morphology, sponge-coral distributions, and sponge-coral identifications are available from the authors. Coral surface area measurements were made by approximation to regular polyhedrons. Corals which immediately fell off the manipulated reefs when sponges were removed constituted <2% of the total corals manipulated (and are not included in the analysis) indicating that any disturbance to the reefs involved in removal of sponges was minimal. Data presented are clumped for all patches over the two observation periods. There was negligible sponge regrowth on manipulated reefs over the experimental period.

The hypothesis that sponges bind corals to the reef frame has previously been considered¹⁴⁻¹⁷: for example, Goreau and Hartman stated that "large encrusting sponges often support and hold such corals in place long after their original holdfasts have been eroded away". To test this hypothesis we removed sponges from patch reefs on the fore-reef slope of Marsagantupo Island, San Blas, Panama, and monitored how the coral survived. The experiments were performed on the leeward side of the island at depths ranging from 13 to 20 m. Marsagantupo Island is exposed directly to the Caribbean Sea. The first 3 months of the experimental period correspond to the dry season in Panama, the period of greatest swell. Although physical data are scant for this region, the Caribbean coast of Panama is regularly exposed to seas up to 10 ft (ref. 18). Eight small patch reefs were selected and all corals mapped as to their position on the patch. All sponges, with the exception of borers and surface encrusters of <1 cm vertical relief, were carefully removed by hand or with a diving knife from half of the reefs. The patches were revisited at three and six months after the removal and all corals remapped.

Figure 1*a* shows the size (surface area live tissue) frequency distribution of foliaceous corals originally extant on unmanipulated reefs and Fig. 1*b* shows that for manipulated reefs. Control reefs lost 2% of their colonies (2% surface area) within three months and 4% of their colonies (3% surface area) after six months; whereas manipulated reefs lost 26% of their colonies (29% surface area) within the first three months and a total of 40% of their colonies (46% surface area) after six months. The high mortality of small colonies, many of which had no direct contact with any sponge, reflects the need of juvenile corals for stable substrata on which to grow. Those small corals which have been asexually reproduced (or which have settled) on top of larger colonies are clearly as dependent on sponge binding as their older counterparts.

The traditional interpretation of the role of sponges in the reef carbonate balance has emphasised the destructive influence of a

small group of biological eroders. Although the influence of biological erosion or the binding capacities of other reef sponges should not be underestimated. Demosponges clearly should be taken into account in the reef carbonate balance equation.

This research was supported by NSF grant OCE 76-23364. Shiptime was provided by the RV Alpha Helix Program and the Smithsonian Tropical Research Institute. K. Rützler provided sponge identifications. We thank L. Fischelson, J. B. C. Jackson, G. Vermeij and C. M. Wahle for their support.

Received 25 June; accepted 21 August 1979.

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Bryozoan overgrowth interactions—the interdependence of competition for space and food

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Space has been demonstrated repeatedly to be a limiting resource to sessile suspension-feeding invertebrates^{1-7,15}, whereas the question of whether food resources are ever limiting has long been a controversial topic^{8,9}. On the basis of previous research and in the virtual absence of any mechanistic understanding, it is commonly assumed that interactions can be interpreted as competition for spatial resources alone. This assumption is clearly unacceptable. Whereas a mobile organism might feed in one location rich in prey, spawn in another safe from predators of its young, and hide in yet another inaccessible to its own predator, a sessile organism cannot separate, spatially or temporally, its demands for food to eat and space on which to live. For the sessile organism, access to food implies access to space and vice versa. Consequently, the empirical observation of competition for space does not justify the assumption that competition for food is unimportant. I report here that competition for food among suspension feeders can occur and may, in fact, provide a mechanism by which sessile organisms compete for space.

Competition between colonial, encrusting organisms occurs whenever the space available on a particular hard substratum is reduced sufficiently for the lateral margins of colonies to come into contact¹⁰. These organisms grow^{11,12} and interact^{1,13-19} along their colony margins exclusively and competition is commonly characterised by overgrowth (Fig. 1*a*). The physical overgrowth of one organism by another is commonly irreversible, resulting in the death of the overgrown tissues^{1,13-19}. In that the overgrowing organism now occupies the space which the overgrown organism had occupied previously, such interactions constitute competition for space²⁰. Although little is known of the mechanisms of overgrowth, they must involve differential growth along interspecific colony margins. The organism that grows fastest here will overgrow its competitor, perhaps by

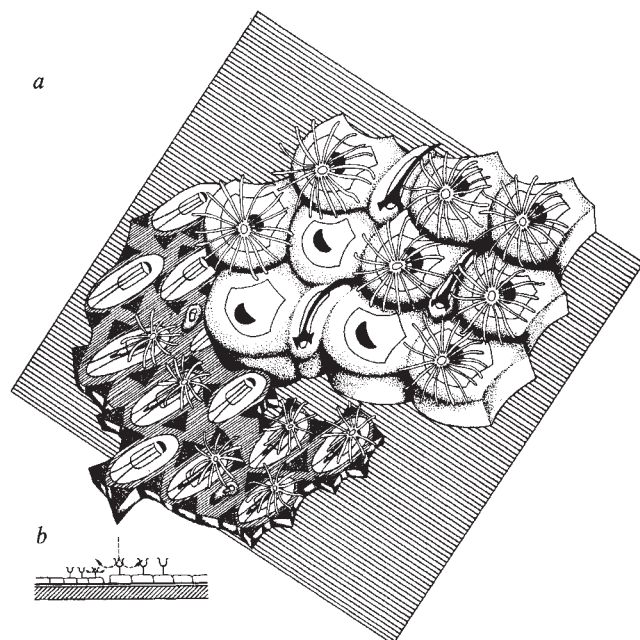


Fig. 1 The overgrowth phenomenon. *a*, Frontal view. In the upper portion *O. alula* has overgrown *A. tincta*, whereas in the lower portion the colony which is growing fastest here will overgrow, that is, that colony which first produces a zooid here. *b*, side view, showing currents and interference effect.

interfering with the access of its competitor to food resources (Fig. 1*a, b*).

Onychocella alula (Hastings) and *Antropora tincta* (Hastings) are two encrusting anascan bryozoans which co-occur on intertidal and shallow subtidal cobble in the eastern Pacific of Panama. The feeding organs of individual zooids (lophophores) extend only a few hundred micrometres above the surface of the colony (*O. alula* and *A. tincta*, respectively: 17 and 12 tentacles, 790 and 410 μm tentacular bell diameter, 320 and 280 μm introvert length, 545 and 295 μm tentacle length), within or very close to the layer of stagnant water adjacent to the substratum^{21,22}. The dominant characteristics of the flow regime surrounding them are those produced by the feeding activities of the organisms themselves^{21,22}. Hence, when in contact, the feeding currents produced by the larger, more robust lophophores of *O. alula* might be expected to interfere with currents produced by adjacent smaller lophophores of *A. tincta* (Fig. 1*b*).

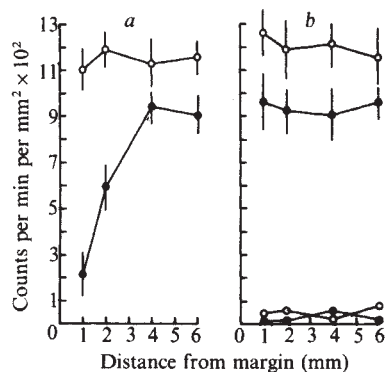


Fig. 2 Radioisotope labelling experiment. Open circles refer to *O. alula* and closed circles to *A. tincta*. Bars are 95% confidence intervals about a mean of 10. Data generated using a piece of lead shaped to the contour of the colonies and impermeable to γ emissions. *a*, Interaction experiment; *b*, bottom lines: rinsing process control; top lines: intracolony effects control.

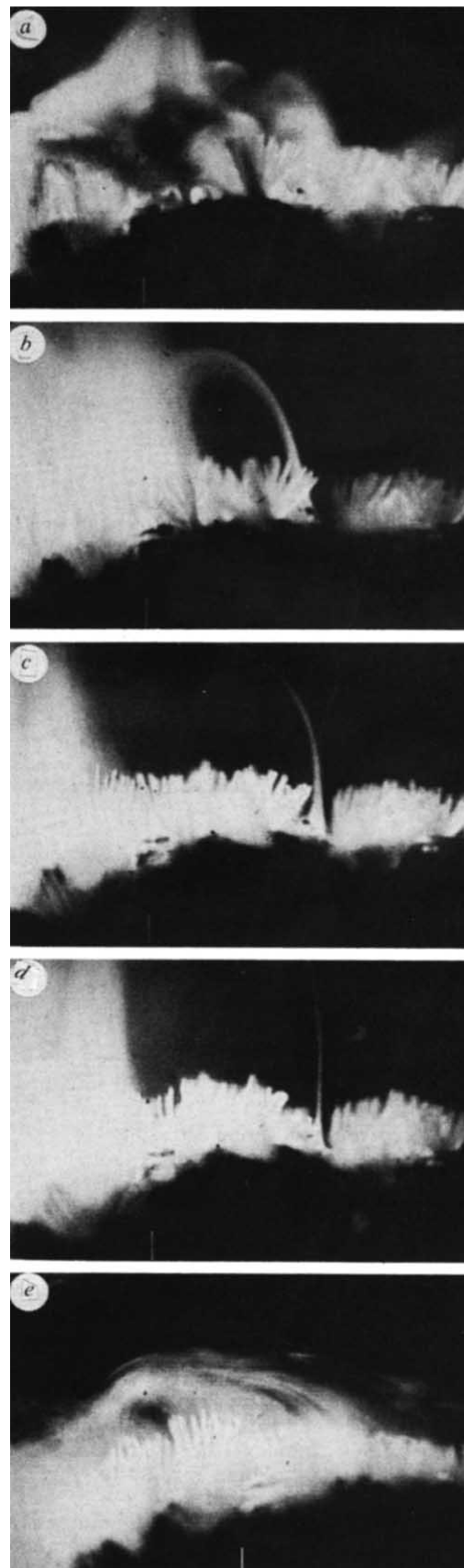


Fig. 3 Microcurrent interference. The point of colony contact is indicated by the bar at the base of all frames. *A. tincta* is the colony to the right of the bar. *a-d*, elapsed time: *a*, 0:00; *b*, 0:19; *c*, 0:45; *d*, 3:32 (min:s). *e*, elapsed time: 0:53. Magnification $\approx \times 60$. See text for discussion.

To test this hypothesis, competing colonies were allowed to feed on radioactive particles, and feeding efficiency (clearance rate) was calculated as a function of the distance from the interspecific contact. Substrata on which *O. alula* and *A. tinctoria* were in contact, but on which overgrowth had not yet occurred, were collected at Punta Paitilla, Panama, and maintained in running seawater at the Smithsonian Tropical Research Institute. Colonies were allowed to acclimate and when feeding, 5 ml of human albumin microspheres (3M Corp., size range 5–90 μm , $\bar{x}$ = 25 μm , s.d. = $\pm 15 \mu\text{m}$, concentration = $5 \pm 2 \times 10^6$) labelled with 5 mCi of ^{99}Tc were introduced into the container. The experiment was aborted if feeding of both colonies was not continuous for the entire 1 h experimental period. Complexities due to possible translocation effects do not occur over this time interval²³. On termination, colonies were killed, rinsed for 1 h under a high-speed jet of seawater, and counted using facilities at the Gorgas Memorial Hospital, Canal Zone. Results are as hypothesised (Fig. 2a); the smaller *A. tinctoria* showed a significant local reduction in clearance rate in contact with *O. alula*, but *O. alula* showed no such reduction. Two control experiments were also carried out. Dead colonies were allowed to 'feed' on labelled microspheres and only a negligible proportion of these remained adherent to colony surfaces after rinsing (Fig. 2b, bottom lines). *O. alula* and *A. tinctoria* were allowed to feed on labelled microspheres in the absence of one another to control for intracolony effects, but no such effect was detected (Fig. 2b, top lines).

The hypothesis that the clearance rate reduction is due to an active interference was confirmed by producing videotapes of interacting colonies. Using a hypodermic syringe, a thin stream of milk-seawater suspension²⁴ was introduced into an experimental container above the interactive margin. The stream was initially disrupted by feeding activity, forming a broad cone of milk (Fig. 3a), which then dispersed to form a cloud above the *O. alula* colony (Fig. 3b). Within seconds, the feeding of *O. alula* filtered the water sufficiently for a zone of cleared water, enclosed by the emerging white band (Fig. 3b, c), to form. This zone expanded and, after several minutes, stabilised over the *A. tinctoria* colony (Fig. 3d), as indicated by the stationary white band. In this region, *A. tinctoria* had access only to water previously filtered by *O. alula*. The width of this zone measured from the interspecific margin to the stationary band was $3.07 \pm 0.24 \text{ mm}$, equivalent to the distance of a clearance rate reduction in the labelling experiment (2–4 mm, see Fig. 2a). In an additional experiment (Fig. 3e), where milk was added above the *A. tinctoria* colony, no zone of cleared water was produced. In contrast to the first experiment, the milk streams away from *A. tinctoria* lophophores to those of *O. alula*. In both cases, *O. alula* interfered with the access of *A. tinctoria* to food resources, that is, competition for food occurred between these suspension feeders.

To overgrow *A. tinctoria*, *O. alula* must grow faster than *A. tinctoria* at the point of colony contact. Unpublished field observations of colonies not in competition have shown that *A. tinctoria* grows faster than *O. alula*, irrespective of colony size. The observation that *O. alula* interferes with the access of *A. tinctoria* to food resources and that this interference effectively reduces *A. tinctoria* food intake suggests that this overgrowth is derived from the mechanism proposed. Competitive interactions for spatial resources may be mediated by competition for food.

This research was supported by NSF grant OCE 76-23364 and ONR contract N00014-75-C-0700. I thank D. Baily, M. Bertness, D. Carlson, F. Carman, B. Graul, J. Jackson, I. Rubino, J. Stokes, C. Wahle, J. Winston, S. Woodin and J. Wulff for their assistance.

Received 23 April; accepted 11 July 1979.

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Action of a nematode-trapping fungus shows lectin-mediated host-microorganism interaction

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Many nematode-trapping fungi capture nematodes using an adhesive present on specific capture organs (for review see ref. 1). Until recently, the mechanism of adhesion was completely unknown. In the case of *Arthrobotrys oligospora*, one of the most common nematophagous fungi, nematodes are trapped in three-dimensional structures of the adhesive network type (Fig. 1a). When a suspension of nematodes is added to an agar culture of the fungus, nematodes are immediately captured and firmly held by the traps. The nematode cuticle is lysed at the point of contact and penetrated by a hypha within one hour². An increased secretion by the fungus of a mucilaginous substance in the presence of prey has been shown by scanning and transmission electron microscopy^{2,3}. Our hypothesis is that the firmness of attachment to the traps despite the struggle of the nematode is due to a series of events, beginning with an interaction between complementary molecular configurations on the nematode and fungal surfaces. We show evidence here for the presence of a lectin on the traps of *A. oligospora* which binds to a carbohydrate on the nematode surface.

Developmentally regulated lectins have been identified in cellular slime moulds, and lectin activity is closely correlated with the development of cohesiveness between the cells^{4–6}. Fungal lectins are produced in the symbiotic relationship between algae and fungi in lichens⁷. The role of lectins in other host-microorganism interrelationships, for example *Rhizobium*-leguminous plant associations, is well documented^{8–11}.

If a lectin-carbohydrate interaction is involved in this relationship, then pre-exposure of the lectin-carrying structure to specific carbohydrates would prevent attachment of the nematodes to the traps. As capture usually takes place within 30 min, microscopic observations at intervals during a 24-h period would give good information on the inhibitory effects of carbohydrates.

To investigate the nematode-trapping ability of *A. oligospora* Fres. (ATCC 24927), the fungus was grown on dialysis

membrane on an agar surface¹². The membranes were transferred to empty Petri dishes before capture of nematodes was tested to eliminate the effects of agar. To test inhibition of capture, cultures of the fungus were flooded with carbohydrate solutions for 24 h before addition of a water suspension of nematodes. Axenically cultivated bacteria-feeding nematodes (*Panagrellus redivivus* Goodey) were used throughout³. Alternatively, nematodes were suspended for 3–24 h in the same carbohydrate solutions, washed and added to untreated fungal cultures.

Flooding of the fungal colony with 20 mM *N*-acetyl-galactosamine (GalNAc) resulted in total inhibition of capture over a 24-h period (Table 1). At this concentration capture was delayed by treatment with melibiose, whereas nematode-trapping ability was unaffected by all other carbohydrates. At higher concentrations of carbohydrates (200 mM) a less pronounced specificity was observed, as maltose, galactose and related sugars prevented capture. These results indicate low specificity of the lectin when exposed to high concentrations of carbohydrate and agree well with reports on the lectins from *Dictyostelium discoideum*^{4,6}. In controls flooded with water or phosphate-buffered saline (0.15 M NaCl, 0.01 M potassium phosphate buffer, pH 7.2, PBS) total capture on dialysis membranes occurred within 30 min–3 h. Conversely, treatment of the nematodes with carbohydrates at 20 and 200 mM failed to inhibit capture. Therefore, the possibility that the carbohydrate-binding activity was located on the nematode was excluded.

The fact that nematode-trapping ability could be suppressed by preincubating the fungus with specific carbohydrates led to the conclusion that a lectin on the fungus is involved in, or responsible for, the interaction. A further consequence of this is that the surface of the nematode must offer suitable binding sites for the lectin, that is GalNAc groups must be present. Two series of experiments tested these hypotheses.

To see if the proposed trap lectin binds to a model prey, the fungus was grown on a piece of dialysis membrane and exposed to an excess of red blood cells (RBC). RBC from different blood groups carry different polysaccharides on their surfaces. Blood group A is characterised by a terminal GalNAc, B carries galactose and blood group O fucose. After incubation for 3–4 h at room temperature the membrane strips were carefully washed in PBS and observed under the microscope.

Significant attachment of RBC took place only at the capture organs (Fig. 1*b*). There was no marked difference between the blood groups, although group A showed a tendency to attach more easily. Thus the proposed trap lectin did not show strict specificity for GalNAc in these *in vivo* tests. As both galactose and fucose had some inhibitory effect on capture of nematodes (Table 1) this low specificity is expected. The developmentally regulated lectins isolated from *D. discoideum* showed a similar low specificity^{4,6}, and various degrees of specificity are reported for other lectins¹³.

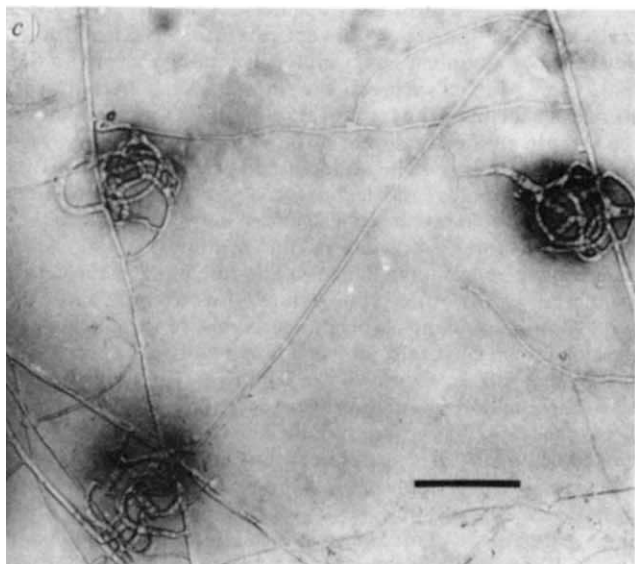
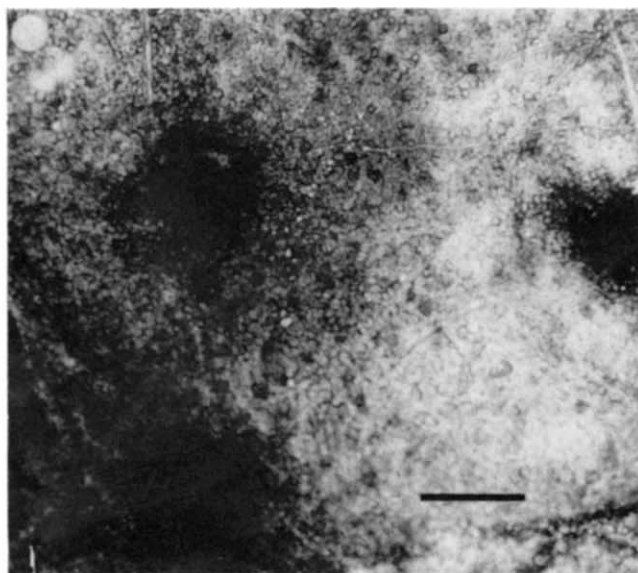
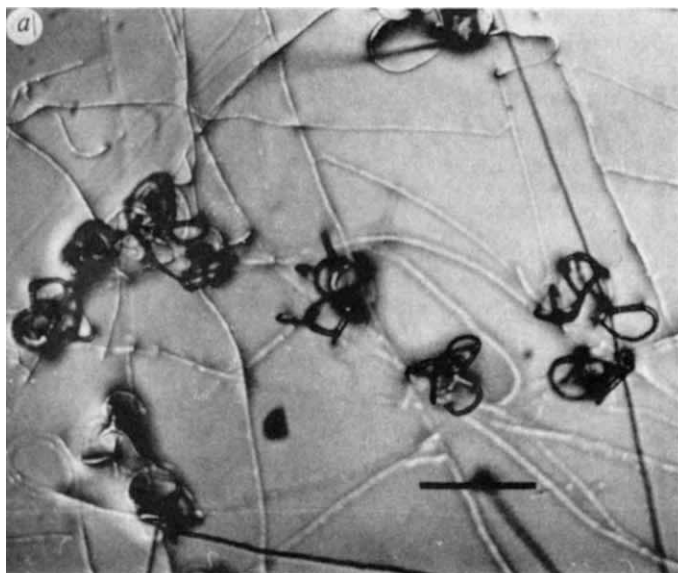


Fig. 1 Attachment of red blood cells to capture organs of *Arthrobotrys oligospora*. Cultures of *A. oligospora* on dialysis membrane were flooded with a suspension of RBC in PBS for 3–4 h. The membrane was then carefully washed twice with PBS and examined in the light microscope, *a*, Three-dimensional traps of *A. oligospora* on agar surface; *b*, RBC of blood group A attached to traps of *A. oligospora*; *c*, the same field of view as in *b* after 10–15 min. The RBC have lysed and the traps have lost turgidity and collapsed. Scale bar 50 μ m.

Table 1 Effect of saccharides on the nematode-trapping ability of *Arthrobotrys oligospora*

Saccharide	Trapping ability at:	
	20 mM	200 mM
N-acetyl-D-galactosamine	—	ND
α -D-galactose	+	—
2-deoxy-D-galactose	+	—
fucose (6-deoxy-L-galactose)	+	(—)
N-acetyl-D-glucosamine	+	ND
α -D-glucose	+	+
2-deoxy-D-glucose	+	+
D(+)-mannose	+	+
α -methyl-D-mannoside	+	+
L-sorbose	+	—
D-fructose	+	+
L-xylose	+	+
D-arabinose	+	—
α -D-melibiose	(—)	—
lactose	+	—
maltose	+	—
D(+)-trehalose	+	+
sucrose	+	+

* The fungus was grown on strips of dialysis membrane¹² placed on the surface of a low-nutrient mineral salts medium¹⁸. Trap formation was induced by addition of a small amount of nematodes to 3–4 d old cultures. Trap-containing strips (7–14 d) were transferred to empty Petri dishes and flooded for at least 20–24 h with the appropriate carbohydrate (20 or 200 mM solution in distilled water or in phosphate-buffered saline). After removal of excess carbohydrate, 1–2 drops of a nematode suspension in water (approx. 1,000 nematodes ml⁻¹) were added to the fungal culture to test capture ability. Microscopic observations of capture were made after 30 min, 3 h and about 24 h. Results of 2–10 different experiments for each carbohydrate. +, normal capture; —, inhibition of capture; (—), capture delayed. ND, not determined.

It was observed that RBC, accumulated around the traps (Fig. 1b), lysed within 1 h. As lysis of RBC occurred to only a small extent spontaneously during these experiments, the effect was attributed to the trap. Immediately after RBC lysis the traps lost their three-dimensional appearance and collapsed. RBC stabilised by crosslinking with glutaraldehyde (80 μ M for 6 h at about 20 °C) were captured by the traps in the same way as untreated cells but did not lyse.

It was not possible to determine the number of RBC bound per unit area or unit weight of fungal biomass in these *in vivo* tests. This was due to differences in trap size and distribution on the dialysis membranes between the experiments and to the irregular shape of the trap structures.

It seems that binding is achieved primarily by the GalNAc structure as inhibition of capture at the lower concentration of carbohydrate occurred only with GalNAc. To demonstrate GalNAc residues on the nematode surface, nematodes were exposed to a peroxidase-lectin conjugate prepared¹⁴ with lectin from *Helix pomatia* with affinity for GalNAc¹⁵. As a blank, the nematodes were exposed to free peroxidase and otherwise treated identically. Incubation of nematodes in substrate (14 mM phenol, 0.8 mM 4-aminoantipyrine, 2 mM hydrogen peroxide) and assay for peroxidase activity¹⁶ according to procedures developed for assay of particle-bound enzymes¹⁷ showed that binding had taken place. Unspecific adsorption of free peroxidase to the nematode or inherent enzyme activity gave some background activity. However, peroxidase activity of conjugate-treated nematodes was approximately six times that of the peroxidase-treated controls. This was interpreted as indicating the presence of GalNAc residues on the nematode surface. Furthermore, on exposing the nematodes to the enzyme-lectin conjugate in the presence of free GalNAc, no activity above the background was observed.

We conclude that a developmentally regulated lectin is present on the traps of *A. oligospora* and binds to carbohydrates on the nematode surface as well as to RBC. The binding initiates

an enzymatic alteration of both nematode and erythrocyte surfaces leading to penetration of the nematode cuticle by a hypha² and lysis of the RBC. The results contribute a new approach to the solution of the problem of specificity in this host-microorganism interrelationship.

We thank Eva Friman for assistance. This work was supported by the Swedish Natural Science Research Council.

Received 17 May, accepted 16 August 1979.

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N-acetyltransferase activity responds to environmental lighting in the eye as well as in the pineal gland

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N-acetyltransferase (NAT), which converts serotonin to N-acetylserotonin, is widely distributed in the vertebrate body^{1,2}. In the pineal gland, however, NAT activity shows circadian changes which respond to environmental lighting both *in vivo* and *in vitro*^{3,4}. NAT is responsible for rhythmic production of the pineal hormone melatonin, which functions in body colour changes⁶, photoperiodic control of reproduction^{7,8}, and behavioural circadian rhythms^{9,10}. The rhythmic changes in NAT have so far been thought unique to the pineal gland. Here we report data for ocular NAT showing a change in a daily light-dark cycle and modification by environmental lighting. The finding of light modified NAT in the eye is significant because it may explain the failure of some investigators¹¹ to find effects of pinealectomy and it may also explain the occurrence of rhythmic melatonin in the blood of some species after pinealectomy^{12–14}. The prerequisites for the production of melatonin exist in the eye as well as in the pineal gland: serotonin¹⁵, hydroxyindole-O-methyltransferase activity¹⁶ and N-acetylindole¹⁷ are present. Furthermore, labelled serotonin is converted to melatonin by the retina *in vitro*¹⁸. Our finding of NAT and its modification by light and dark completes the argument for ocular capacity for melatonin production in a cyclic manner modified by environmental lighting.

In our experiments the eyes and the pineal gland were investigated in parallel by assaying both structures from animals during light-dark treatments. The data in Fig. 1 (and in two to four replicate experiments) illustrate the following statistically significant results ($P < 0.05$): (1) In a light-dark cycle, dark-time NAT was 7.0–11.8 times as high as light-time NAT in the pineal gland; dark-time NAT was 2.7–2.9 times light-time NAT in the eye. (2) When chicks were placed in constant dark (DD), pineal NAT in their subjective dark-time was 3.7–5.9 times that in the

Table 1 Ocular NAT in four species was measured in the light and dark times of a light-dark cycle (LD12:12)

	N-acetyltransferase activity (nmol per eye per h)	
	Light	Dark
Chick, <i>Gallus domesticus</i>	3.038 ± 0.122	8.726 ± 0.898
Rat, <i>Rattus norvegicus</i>	0.475 ± 0.066	1.322 ± 0.148
Sparrow, <i>Passer domesticus</i>	0.119 ± 0.029	0.483 ± 0.053
Hamster, <i>Mesocricetus auratus</i>	0.726 ± 0.148	0.527 ± 0.132

Values are means ± s.e.m., $N = 4$.

light-time; in contrast, in the eye, subjective dark-time NAT was not significantly different from subjective light-time NAT. (3) When the chicks were placed in constant light (LL) subjective pineal dark-time NAT was suppressed (to 25–27% of its LD value); ocular dark-time NAT was likewise suppressed (to 35–38% of its LD value). (4) NAT shows a 'rapid response' to light during the dark-time^{19,20}. Chicks were exposed to light for 1 h during their subjective dark-time which suppressed dark-time pineal NAT (61–76%) and also suppressed ocular NAT (36–59%).

The specific activity of NAT in the pineal gland was up to three times that in one eye during the dark-time. Table 1 shows data for four species kept in light-dark cycles. The chick, the sparrow, and the rat all had ocular dark-time NAT three to four times higher than ocular light-time NAT. The data for ocular NAT and its modulation by environmental lighting are convincing evidence that the pineal gland is not singular in its ability to metabolise serotonin and produce melatonin in a rhythmic fashion.

In the experiments reported here—two-point experiments—there was no evidence that the ocular NAT has a persistent circadian rhythm in the same conditions where such a rhythm clearly occurs in the pineal gland. Nor did the eye of the hamster have an NAT rhythm in the LD cycle. However, these two-point experiments are not conclusive evidence that there is no circadian rhythm—it is possible to miss a narrow peak of activity even with more frequent time points.

When NAT activity in the two eyes was compared the values were positively correlated ($r = 0.82$ calculated for six pairs from LD chicks). In the experiments detailed in Fig. 1 and Table 1, NAT was measured in the neural retina plus the pigmented layer plus the accompanying wetting vitreous humor. In a separate experiment done to partition the NAT activity (Table 2), 41–56% of NAT was localised in the neural retina and 42–57% was in the pigmented layer; only 2% was in the 10- μ l sample of vitreous humor (used to estimate the accompanying wetting of the neural retina and pigmented layers by humor during the dissection). As whole eyes were not analysed, the measurements do not represent the total ocular melatonin synthesising capacity. Bubenik *et al.*¹⁷ localised *N*-acetylindole to the outer layer of the neural retina.

To make physiological sense of the relative contributions of pineal and ocular melatonin requires knowledge of the target(s) and functions of melatonin. Melatonin synthesis by the eye

Table 2 NAT was measured separately in the three portions of the eye which were used together for the experiments in Fig. 1 and Table 1

	N-acetyltransferase activity	
	Light	Dark
Eye		
Vitreous humor	0.070 ± 0.027	0.144 ± 0.065
Neural retina	1.654 ± 0.270	9.678 ± 1.157
Pigmented layer	2.304 ± 0.350	7.409 ± 0.777
Pineal gland	2.632 ± 0.451	22.099 ± 4.374

Each value is a mean ($N = 4$) ± s.e.m. NAT is given as nmol per tissue per h for the neural retina and pigmented layer (one eye), as nmol per gland per h for the pineal gland, and as nmol per 10 μ l per h for the vitreous humor.

could be an endocrine function, or, alternatively, the eye itself may be the target. For endocrine function, the eye may be an additional source of melatonin which acts in the circulation as a hormone augmenting pineal production.

There are several hypothetical roles for melatonin within the eye itself. Ocular melatonin may function in the daily rhythms reported for outer disk shedding in the retina²¹. Two types of

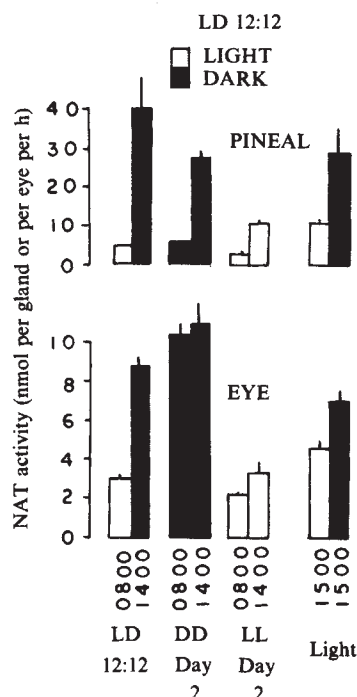


Fig. 1 NAT activity in the eyes and pineal glands of chicks kept in a light-dark cycle (LD), constant dark (DD) or constant light (LL). Chicks were kept in a light-dark cycle, LD12 h : 12 h, lights-off 1000, from day 1 after hatching until they were 23 days of age. The chicks were then divided into groups and kept for 2 more days in either the same LD cycle, DD or LL. On the second day they were killed at 0800 (subjective day-time) or at 1400 (subjective night-time). A fourth group of chicks were kept in the LD cycle but exposed to light for one hour (1400–1500) during their subjective dark-time—this group and controls kept in the dark were killed at 1500. The data shown here are from one of two replicate experiments ($N = 4$ chicks per group, bars represent mean values ± s.e.m.); the LD experiment was performed four times. For these experiments the combined neural retina plus pigmented layer was dissected from the eyes immediately after the chicks were killed. The ocular tissue and pineal glands were then quick frozen on dry ice and assayed for NAT within a week⁵.

movement have been noted in the eyes of lower vertebrates and some birds: retinal cones elongate and shorten²², and pigment migration is thought to occur in the melanin containing cells²³. The melanin pigment layer widens in the dark and narrows in the light—pinealectomy abolishes the dark displacement of pigment, melatonin increases the displacement²³. The cones shorten in the light and lengthen in the dark—short-term effects which are superimposed on an underlying circadian rhythm which persists in constant light or constant dark^{22,24}.

We conclude that the eye is capable of rhythmic melatonin synthesis (in the chick, sparrow and rat), and that the chick ocular NAT is modified by environmental lighting (constant light, dark-to-light transitions). It is probable that ocular melatonin gets into the bloodstream, because some pinealectomised animals have rhythmic melatonin circulating after pinealectomy^{12–14}.

This investigation was supported by NSF grant PCM 77–26474 to S.B. and a Faculty Research Award to S.B. from Temple University. We thank Joel Sheffield, Beth Bernside and Sue Klein for their assistance.

Note added in proof: Hamm and Menaker (personal communication) have evidence that the ocular NAT rhythm is circadian with a later and narrower peak on the second day of constant dark; we now have evidence for circadian clock involvement from light-to-dark transition experiments.

Received 3 May; accepted 14 August 1979.

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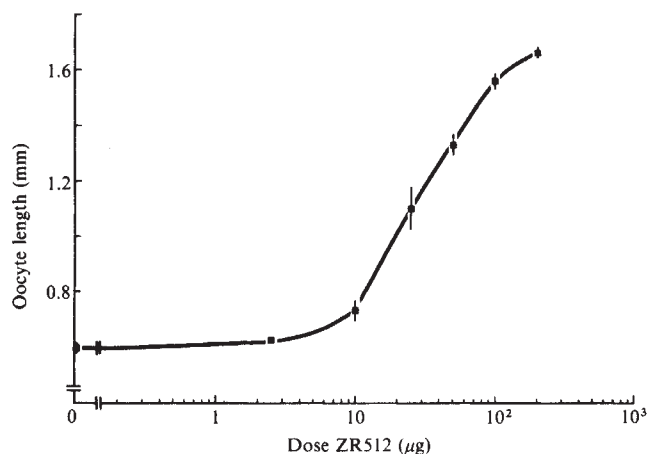


Fig. 1 Basal oocyte length on day 6 after treatment of allatectomised female *D. punctata* with ZR512. Oocytes attained mature length with the 200-μg dose. Each point represents the mean $\pm$ s.e.m. of determinations on 5–10 animals.

weight of these females was 190 ± 3 mg. Figure 1 shows a semi-logarithmic plot of ZR512 dose against the basal oocyte length 6 days after analogue application. At 2.5 μg the oocyte response was slightly greater than that of the controls but not significantly different; between 10 and 100 μg it was virtually linear; at 200 μg the oocytes attained maximum mature length (1.65 mm) with 80% chorionated, ready for oviposition. Oviposition was elicited in 6 of the 7 females treated with 200 μg and in 5 of the 9 females receiving 100 μg ZR512. None of the females treated with acetone ($n = 9$), 25 μg ($n = 10$), or 10 μg ($n = 5$) ZR512 oviposited. From these observations we conclude that a single topical application of ZR512 (≥ 100 μg) in acetone effectively mimics the action of JH in *Diploptera punctata*, permitting both oocyte maturation and oviposition in the absence of authentic hormone.

To test the effects of this analogue on the rate of JH synthesis by the CA, normal mated females were treated with various doses of ZR512 between 1 and 5 h after adult emergence. Every day for six days after the treatment, the length of the basal oocytes was recorded and the rate of C_{16} JH (JH III) synthesis by the CA was assayed^{4,5}. Figure 2a shows the progress of egg growth for 100, 25 and 2.5-μg doses of ZR512, and acetone-treated controls. The rate of oocyte growth showed a positive correlation with the concentration of ZR512 applied. The difference between oocyte lengths in 25 μg- and 100 μg-treated animals and controls is significant for days 2–6 ($P < 0.001$; Students *t*-test), whereas in animals treated with 2.5 μg, although the oocytes were larger than controls on days 2–5, only on day 4 is the difference significant ($0.02 < P < 0.01$). These results indicate that exogenously applied analogue may act with JH released by the CA to raise the titre of JH–JHA such that eggs grow more rapidly in treated animals than in controls. (For example, the control females had 1.33 mm-long oocytes on day 5 whereas this length was achieved at 4.25, 3.25 and 2.75 days in females treated with 2.5, 25 and 100 μg of analogue respectively.) Alternatively, the ZR512 may stimulate the CA to synthesise JH at a greater rate, causing an elevation in JH titre.

The choice between these hypotheses is facilitated by the *in vitro* determination of the rate of JH synthesis by the CA of the same animals for which egg growth is shown (Fig. 2a). These measurements provide an estimation of the contribution of endogenous JH towards the increased rate of oocyte growth (Fig. 2b). At 100 μg ZR512, the normal cycle of JH synthesis was depressed, reaching a maximum rate of only 28 pmol per h per pair on day 3 and minima on days 2 and 5 of 6–7 pmol per h per pair. Animals receiving 25 μg of ZR512 showed rates of JH synthesis similar to those of acetone controls on days 2–4 but depressed rates thereafter (maximum rate of JH synthesis 54 pmol per h per pair). Maximal rates of JH synthesis (87 pmol

Modulation of juvenile hormone synthesis by an analogue in the cockroach

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The juvenile hormones (JH) are novel insect sesquiterpenoid products synthesised by paired endocrine glands known as the corpora allata (CA). These glands are innervated by neural and neurosecretory elements originating in the central nervous system (CNS)^{1–3} and are known to undergo predictable changes in synthesis and release of JH during the reproductive cycle^{4–8}. This implies that the CA are regulated by intrinsic physiological signals. For example, in the viviparous cockroach, *Diploptera punctata*, if the haemolymph titres of JH are experimentally altered by removal of one member of the pair or by implantation of additional CA, the original CA undergo appropriate corrective responses in JH synthesis^{9,10}. This is partly due to signals from the CNS because denervation of the CA attenuates the compensatory change¹⁰. The depression in JH synthesis after addition of exogenous JH to the system has also been observed^{11,12}. However, there have been no direct measurements of JH synthesis following treatment with exogenous JH analogues *in vivo*. We show here that in *Diploptera punctata*, topical application of a JH analogue (JHA) depresses the synthesis of JH at high doses but stimulates synthesis at low doses and that the analogue can support oocyte growth and maturation in the absence of the CA and that application to intact animals stimulates oocyte growth rates. These results indicate that the analogue can replace authentic JH.

Mated females of *Diploptera punctata* were allatectomised between 1 and 5 h after adult ecdysis⁹. Three to four days later, the JH analogue ZR512 (ethyl (2E)-3,7,11-trimethyl-2,4-dodecadienoate, Zoecon) was applied in 5 μl of acetone to the dorsal surface of the abdomen (wings were removed). The mean

per h per pair) by CA of control animals were observed on day 6. On the basis of oocyte lengths attained by this time (Fig. 2a) and from control measurements reported elsewhere^{9,10}, this value probably represents the peak of the normal cycle. In sharp contrast to the depression of JH synthesis by 100 and 25 µg doses of ZR512, animals receiving 2.5 µg of ZR512 showed a marked stimulation in JH synthesis on day 4 (126 against 49 pmol per h per pair) ($P = 0.028$). Similar stimulation on day 4 has been observed after treatment with 2.5 µg C₁₆ JH (unpublished data). We conclude that the high doses of ZR512 depress synthesis of JH, whereas the low dose stimulates synthesis. The accelerated oocyte growth observed at high doses of ZR512 seems to be due to the cumulative action of the analogue and endogenous JH on the oocytes causing an elevation in the titre of JH-active compounds.

To account for these opposing actions of the applied hormone analogue, we hypothesise that below a certain dose, JH synthesis is stimulated by the analogue, (that is, shows positive feedback) and that at a higher dose, a negative feedback operates to depress JH synthesis. Such a hypothesis would also account for the increase in rates of JH synthesis after unilateral allatectomy and the suppression of JH synthesis in the presence of supernumerary CA^{9,10} and for the rapid changes in JH synthesis during the normal reproductive cycle⁵. For example, a positive feedback loop may be responsible for the rapid increase in JH synthesis observed in normal females between days 3 and 5 after emergence⁵. Such loops have been proposed for insect endocrine systems, particularly for the interaction between the CA and cerebral neurosecretory cells¹³⁻¹⁶ and between the prothoracic glands and cerebral neurosecretory cells¹⁷⁻¹⁹. However,

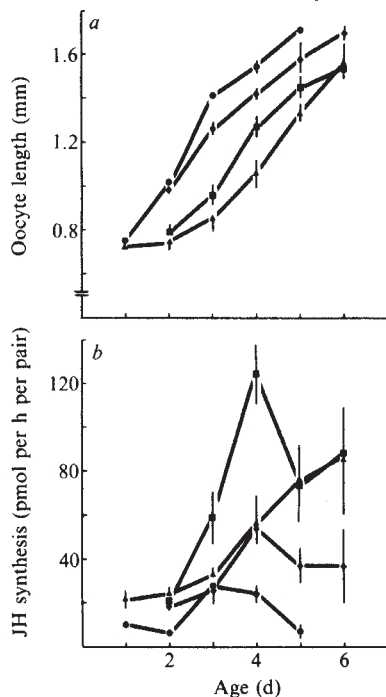


Fig. 2 The effect of ZR512 on oocyte growth and JH synthesis in *D. punctata*. Animals were treated topically on day 0 and assayed on days 1–6. *a*, Mean oocyte lengths $\pm$ s.e.m.; *b*, mean rates of JH synthesis $\pm$ s.e.m. $n = 5$, except for day 4 where $n = 10$ and day 5 where $n = 10$ for 2.5 µg, $n = 9$ for 25 µg and acetone and $n = 7$ for 100 µg. Rates of synthesis of C₁₆JH were determined using an *in vitro* radiochemical assay which uses the incorporation of the methyl moiety of [¹⁴C-methyl]methionine into C₁₆JH. Individual pairs of CA were incubated for 3 h at 28°C in medium 199 (Hanks' salts; with glutamine, HEPES buffer, 25 mM, pH 7.2-GIBCO) plus Ficoll (20 mg ml⁻¹) to which [¹⁴C-methyl]methionine (Amersham-Searle, final specific activity 35–39 mCi mmol⁻¹) was added. Procedures for the preparation of the incubation medium, and the separation and quantitation of biosynthesised radiolabelled JH have been described previously⁴⁻⁵. ●, 100 µg ZR512; ◆, 25 µg ZR512; ■, 2.5 µg ZR512; ▲, acetone.

definitive proof for such pathways in insect systems has been lacking, although they are well known in vertebrate systems—for example the preovulatory surge of luteinising hormone which occurs as a result of increasing titres of estradiol²⁰. It is significant that in *D. punctata* the proposed positive feedback loop responsible for the stimulation of JH synthesis in animals treated with 2.5 µg ZR512 operates only for a short period during the reproductive cycle, with a maximum effect on day 4. This is precisely the time at which rates of JH synthesis are rapidly increasing in control animals⁵. This suggests the existence of a temporal gate in the centre which regulates the CA—thus the regulatory centre may be sensitive or responsive to increasing titres of JHA only for a brief period during the reproductive cycle.

These results also demonstrate that high doses of JHA suppress JH synthesis by the CA. Implantation of supernumerary CA^{9,10}, as a source of JH, as well as topical treatment with C₁₆JH (unpublished data) also result in a reduction in JH synthesis. The simplest explanation for this suppression is a negative feedback loop. Such mechanisms are well known in the regulation of endocrine gland function.

These feedback signals may operate on an intermediate regulatory centre outside the CA but it is conceivable that the signals may also feed back directly on the CA. However, Pratt and Finney²¹ did not observe inhibition of JH synthesis by C₁₆ JH *in vitro* in *Periplaneta americana*. On the other hand, we have shown that isolation from the regulatory centres of the CNS by denervation of the CA attenuates but does not totally abolish corrective responses by the CA^{9,10} and, although this may suggest a feedback loop operating directly on the CA, it is equally likely that a regulatory centre outside the CA may be controlling the glands via a humoral pathway.

Although the mode of action of ZR512 still remains to be defined precisely, it is clear that, at high doses, it can be regarded as a suppressor of JH synthesis. Using the *in vivo-in vitro* assay described here, it is now possible to screen JH analogues for such activity. Those analogues which suppress CA activity but have no gonadotrophic activity may have potential as insect control agents. The only compounds known to have such activity are the precocenes^{22,23} but their mode of action is unclear.

Our assay differs from other screening procedures²¹⁻²⁴ in that it permits a simultaneous assessment of analogues as inhibitors of both JH synthesis and ovarian growth in the same insect. The techniques described here should also be valuable for investigating the role of the centres outside the CA in regulating these endocrine glands.

This study was supported by the Natural Sciences and Engineering Research Council of Canada. We thank Dr G. Staal for ZR512.

Received 1 June 1979; accepted 10 August 1979.

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FSH stimulates hyaluronic acid synthesis by oocyte-cumulus cell complexes from mouse preovulatory follicles

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A surge in the secretion of gonadotropins stimulates several processes in mammalian preovulatory follicles, including oocyte maturation, cumulus expansion (mucification) and ovulation. This surge results in increased circulating levels of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH). It has generally been thought that LH is primarily responsible for the stimulation of the maturational processes in preovulatory follicles that results in ovulation and corpus luteum formation, and that FSH stimulates the development of the next wave of preovulatory follicles. A direct role for FSH in preovulatory events has been unclear. However, two studies have suggested that FSH is essential for certain preovulatory processes. First, there is evidence that the production of plasminogen activator by granulosa cells is more sensitive to FSH than to LH¹. Plasminogen activator could convert follicular fluid-borne plasminogen to plasmin, a protease capable of weakening the follicle wall². Second, highly purified FSH, but not highly purified LH, stimulates cumulus expansion by isolated mouse oocyte-cumulus cell complexes³. A critical step in cumulus expansion is the deposition of a hyaluronic acid matrix, so that the synthesis of this glycosaminoglycan is very important in the preparation of

the mouse oocyte-cumulus cell complex for normal ovulation. I report here that the synthesis of hyaluronic acid *in vitro* is stimulated by FSH but not by LH.

Biological grade preparations of FSH and LH stimulated hyaluronic acid synthesis by isolated mouse oocyte-cumulus cell complexes in a dose-dependent fashion (Fig. 1a). However, the complexes were much more sensitive to FSH than to LH, suggesting that the response to biological grade LH was due to contamination with FSH. To test this idea, the complexes were incubated in highly purified rat FSH and LH. The former, but not latter, significantly ($P < 0.01$) stimulated hyaluronic acid synthesis (Fig. 1b). Similarly, highly purified human chorionic gonadotropin (HCG), which competes for the same binding sites as LH⁴, did not stimulate hyaluronic acid synthesis (Fig. 1b). As crude HCG significantly ($P < 0.01$) stimulated the synthesis of hyaluronic acid (Fig. 1b), this preparation is probably contaminated with chorionic FSH. Treatment of FSH-stimulated complexes with *Streptomyces* hyaluronidase indicated that 90% of the ³H-glucosamine incorporated into cetylpyridinium chloride-precipitable counts was incorporated specifically into hyaluronic acid (Fig. 1b). Dibutyl cyclic AMP, theophylline and cholera toxin all significantly ($P < 0.01$) stimulated hyaluronic acid synthesis (Fig. 1c).

It is concluded that FSH, and not LH, stimulates the synthesis and deposition of hyaluronic acid by isolated mouse oocyte-cumulus cell complexes. Therefore, FSH plays a direct, critical role in an important event, hyaluronic acid synthesis, that is essential for the cumulus expansion preceding normal ovulation. This is the first demonstration of a specific biosynthetic process, related to the ovulatory process, that is stimulated directly by FSH and cannot be directly stimulated by LH. The finding that dibutyl cyclic AMP, theophylline (an inhibitor of cyclic AMP degradation) and cholera toxin (a stimulator of cyclic AMP synthesis) all stimulated hyaluronic acid synthesis suggests that the action of FSH is probably mediated by cyclic AMP.

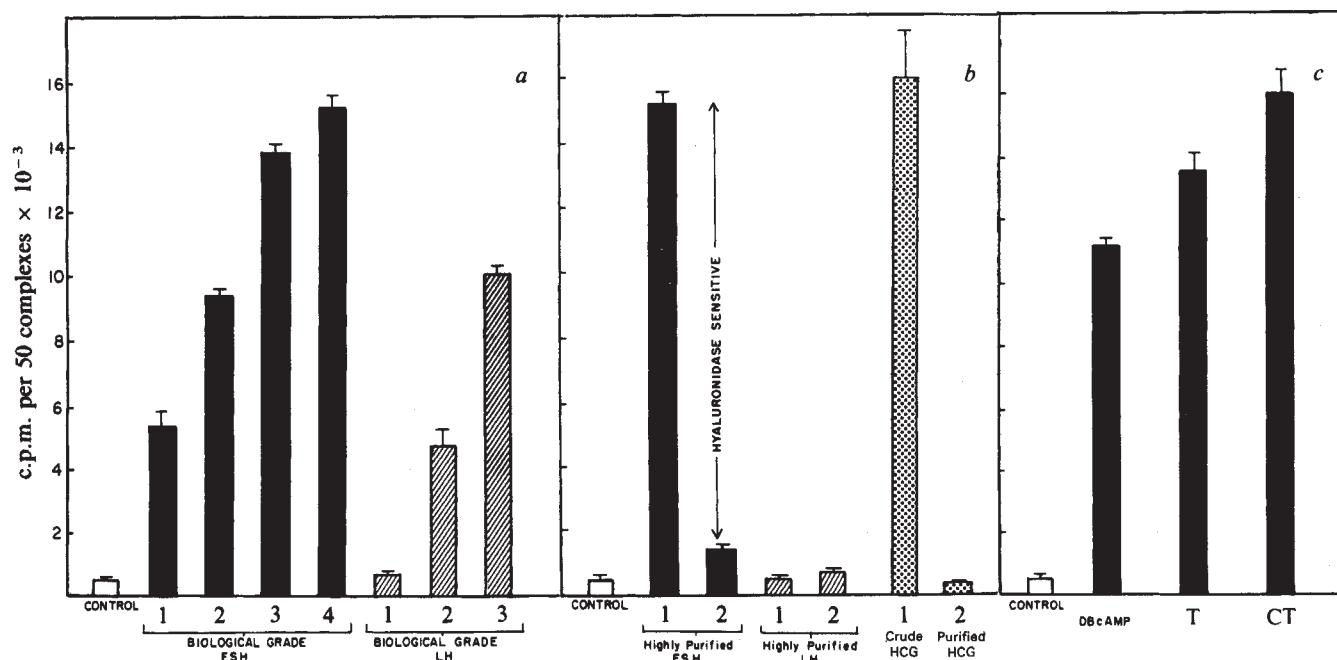


Fig. 1 (C57BL/6J × LT/Sv)_{F1} mice, 26–28-d-old, were injected with 5 IU of pregnant mare serum gonadotropin. Forty-eight hours later the large graafian follicles were punctured with 25-gauge needles and the oocyte-cumulus cell complexes were removed and washed by serial passage, using micropipettes, through four dishes containing 2.0 ml of Whitten's medium⁶ supplemented with 5% fetal bovine serum (Sterile Systems Inc.). Hormones and isotope were added to a culture dish containing 2.0 ml of medium and incubated for 17 h at 37 °C in an atmosphere of 5% O₂, 5% CO₂ and 90% N₂. The following hormones were added at various concentrations: a, biological grade FSH (NIAMDD-rat FSH-B1), 0.1 µg ml⁻¹(1), 0.5 µg ml⁻¹(2), 1.0 µg ml⁻¹(3), 5.0 µg ml⁻¹(4); biological grade LH (NIAMDD-bovine LH-B10), 1.0 µg ml⁻¹(1), 5.0 µg ml⁻¹(2), 10.0 µg ml⁻¹(3); b, highly purified FSH (NIAMDD-rat FSH-I-4), 1 µg ml⁻¹(1, 2); highly purified rat LH (NIAMDD-rat LH-I-4), 1 µg ml⁻¹(1), 5 µg ml⁻¹(2); crude HCG (Sigma), 5 IU ml⁻¹; highly purified HCG (R. E. Canfield preparation CR-119), 5 IU ml⁻¹; c, dibutyl cyclic AMP, 5 mM (DBcAMP, Sigma); theophylline 5 mM (T, Sigma); cholera toxin 2 µg ml⁻¹ (CT, Sigma). Hyaluronic acid synthesis was measured exactly as described by Solursh⁷, in groups of 50 oocyte-cumulus cell complexes incubated in medium containing 25 µCi ml⁻¹ of D-[6-³H]glucosamine hydrochloride, specific activity 38 Ci mmol⁻¹ (Amersham) for 17 h. Specificity of isotope incorporation into hyaluronic acid was determined by sensitivity of the labelled material to highly specific *Streptomyces* hyaluronidase⁸. After the 17-h labelling period some groups of the washed complexes were incubated in 5 IU ml⁻¹ *Streptomyces* hyaluronidase (Calbiochem) at 37 °C for 3 h and then assayed according to Solursh⁷. At least three groups of 50 complexes were assayed for each group. Data are expressed as c.p.m. per 50 complexes ± s.e.m., and were subjected to one-way analysis of variance followed by the Student-Newman-Keuls multiple range test.

Although LH is ineffective in stimulating hyaluronic acid synthesis *in vitro*, an indirect role for LH in regulating cumulus expansion *in vivo* cannot be excluded. The follicular fluid of preovulatory follicles contains significant amounts of FSH before the LH surge⁵, but this FSH does not stimulate cumulus expansion before the gonadotropin surge. Perhaps LH affects the preovulatory follicle in some way that permits the oocyte-cumulus cell complex to respond to FSH and synthesise hyaluronic acid.

This research was supported by NSF grant PCM 76-03047. I thank Dr A. F. Parlow and the Pituitary Hormone Distribution Program of the NIAMDD for supplying the pituitary hormones, Dr R. E. Canfield and the Center for Population Research of the NICHD for providing the purified HCG, and Cindy Grindle for research assistance.

Received 9 July; accepted 2 August 1979.

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Unmasking of fetal determinants on adult bone marrow cells

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The phasing-out of an SV40 cross-reactive fetal antigen in the developing hamster fetus after day 10 occurs concomitantly with an increase of bound sialic acid in membrane, microsomal and soluble cell fractions¹. A masking phenomenon is suggested by the fact that the antigen can be uncovered on older fetal tissue by trypsinisation². Similarly, a murine oncofetal antigen under study in our laboratory is optimally expressed on the haematopoietic stem cells of the fetal liver at the 15th day of gestation³, just before the onset of haematopoiesis and collagen production in the spleen⁴. The expression of this antigen on human leukaemic blast cells has, furthermore, been correlated with low levels of sialic acid on the surface of these tumour cells⁵. As fetal determinants can be expressed on adult bone marrow in disease states not involving malignant transformation⁶, it is of interest to know whether production of this oncofetal antigen is repressed, or whether the determinants are in cryptic form at the plasma membrane due to some masking phenomenon. The investigation described here demonstrates that determinants which cross-react immunologically with fetal liver tissue can be enzymatically unmasked on normal adult murine haematopoietic stem-cell surfaces. This finding suggests that the expression of these oncofetal antigens on malignant cells does not depend on *de novo* production of the antigens, and that these determinants may play some part in normal differentiation control mechanisms.

We used adult bone marrow (ABM) cells from the femurs of 16-week-old male mice and 15-day fetal liver (FL) cells from

fetuses of primigravida BALB/c and C57BL/6 mice. Following fractionation on a discontinuous albumin gradient to isolate the haematopoietic stem-cell fractions⁷, ABM or FL stem cells were incubated individually or in sequence with collagenase (Calbiochem; grade A) and neuraminidase (Behring, from *Vibrio cholerae*) at 37°C for 30 min per enzyme. Cells were washed three times after each incubation. Collagenase incubation and washes were carried-out with 0.05 M Tris-HCl buffer, pH 7.6, containing 0.005 M CaCl₂. Neuraminidase buffer, pH 7.2, contained 0.004 M Na acetate, 0.116 M NaCl and 0.005 M CaCl₂. Enzyme incubations were carried out with constant mixing of cell suspensions on a Junior Orbit bath shaker at 200 r.p.m.

Subsequently, the enzyme-treated target (ABM and FL) cells were incubated with normal rabbit serum or with a rabbit antiserum which was raised against BALB/c FL cells (10⁷ cells, subcutaneously, weekly for 10 weeks) denoted as rabbit anti-mouse fetal liver serum. After appropriate absorptions, this latter antiserum did not react with adult murine spleen, liver or ABM cells. Indirect immunofluorescence was then carried out using a previously described method⁸. Cells for the electron microprobe (X-ray fluorescence) analysis were reacted with ferritin-tagged antibody or normal globulin. Single cells were analysed in a modified Hitachi HU 10A transmission electron microscope which was adapted with a high resolution lithium-drifted silicon (Si(Li)) detector. Computer analysis of the intensity of the K α peak for iron (6.4 KeV) determined the number of ferritin-tagged antibody molecules bound by cell membrane determinants^{8,9}. The quantitative expression of fetal antigen determinants was correlated to the respective enzyme treatment(s).

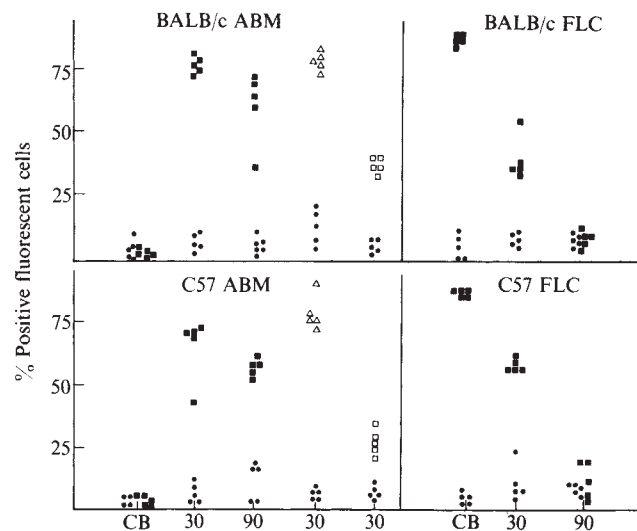


Fig. 1 Enzymatic modulation of cell-surface expression of fetal antigen determinants assayed by indirect immunofluorescent labelling. Mouse ABM or FL cells were treated with control buffer (CB) or with 30 or 90 U collagenase, followed by 12.5 U neuraminidase, and then incubated with normal rabbit serum (●), rabbit anti-mouse fetal liver serum (■), antiserum absorbed with adult mouse tissue (△) or antiserum absorbed with mouse fetal liver (□). Each point represents one experiment evaluating 100 cells.

Figure 1 demonstrates the effect of sequential incubations of ABM cells with collagenase and neuraminidase. The optimal regimen for uncovering fetal determinants on BALB/c ABM cells was 30.0 U collagenase followed by 12.5 U neuraminidase. After this enzyme treatment 80% of the ABM cells were labelled by rabbit anti-mouse fetal liver serum. There was no

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Table 1 Quantitation of membrane-associated fetal determinants

BALB/c target cell*	Enzyme treatment†	Serum*	No. of fetal determinants per cell (by X-ray)‡	% Positive fluorescent cells X-ray	Indirect IF‡
FL	None	RAMFLS	$1.2 \pm 0.6 \times 10^6$	96	89 ± 2
FL	None	NRS	$1.0 \pm 0.05 \times 10^4$	8	4 ± 3
ABM	None	RAMFLS	$1.6 \pm 0.05 \times 10^4$	18	4 ± 3
ABM	None	NRS	$0.9 \pm 0.05 \times 10^4$	15	4 ± 4
ABM	N/C	RAMFLS	$1.0 \pm 0.2 \times 10^4$	18	19 ± 2
ABM	N/C	NRS	$1.3 \pm 0.2 \times 10^4$	12	8 ± 2
ABM	C/N	RAMFLS	$1.6 \pm 0.5 \times 10^5$	74	80 ± 4
ABM	C/N	NRS	$1.2 \pm 0.4 \times 10^4$	22	8 ± 3
ABM	C/N	RAMFLS (absorbed FL)	$0.8 \pm 0.03 \times 10^4$	28	39 ± 3

* ABM Adult bone marrow; FL, fetal liver; RAMFLS, rabbit anti-mouse fetal liver serum; NRS, normal rabbit serum.

† Target cells were incubated with 30 U collagenase (C) and 12.5 U neuraminidase (N), each for 30 min at 37°C, in the sequence indicated.

‡ Mean $\pm$ s.d.; $n = 50$ for X-ray fluorescence, assays done one cell at a time; $n = 100$ for immunofluorescence (IF), assays repeated five times.

increase in nonspecific labelling of cells by normal rabbit serum. C57BL/6 ABM cells reacted in the same manner, showing that detection of the fetal determinants was not restricted to H-2^d allelic expression. The reverse enzyme sequence or treatment with either enzyme alone did not increase the reactivity of the ABM cells with the rabbit antiserum.

The rabbit anti-fetal serum was absorbed twice with FL cells (10^7 cells ml⁻¹, 1 h at 37°C, 16 h at 4°C), and then incubated with syngeneic (either BALB/c or C57BL/6) ABM cells which had been subjected to the optimal enzyme regimen. As shown in Fig. 1, the number of unmasked ABM cells labelled by the absorbed serum fell by 50% for BALB/c and by 67% for C57BL/6 cells. Specificity controls showed that absorption with other adult cells (spleen, liver, intestine or thymus) produced no reduction in labelling of unmasked ABM by the antiserum. Furthermore, these tissues were not labelled by the antiserum after similar enzyme treatment.

As demonstrated in Fig. 1, rabbit antiserum reacted optimally with fetal liver cells which were not enzymatically modified (90% fluorescent cells). The incubation of FL cells with collagenase followed by neuraminidase resulted in a decrease in the number of cells labelled by the antiserum as the concentration of collagenase was increased. In contrast to ABM cells, fetal antigen expression on FL cells could be modified by treatment with either enzyme alone.

X-ray immunofluorescence spectrometry has allowed measurement of the rabbit antiserum-reactive determinants on the cell surface (Table 1). Fetal determinants expressed on untreated FL cells averaged 10^6 determinants per cell. ABM cells at the optimal conditions for unmasking displayed approximately an order of magnitude fewer determinants than the FL cells. Untreated ABM cell controls or ABM cells treated with neuraminidase followed by collagenase displayed approximately 10^4 determinants per cell (2 orders of magnitude below untreated FL cells). Table 1 also presents data comparing the number of cells which displayed significant antibody binding (>95% confidence) as determined by X-ray fluorescence with the number of positive cells in the indirect immunofluorescence assay. Although the reactivity of anti-fetal serum for unmasked ABM was not completely abolished in the indirect fluorescence

assay after absorption with FL cells, X-ray analysis revealed that the percentage of positive cells and the number of fetal determinants per cell labelled by the absorbed antibody were comparable to background levels with normal rabbit serum.

The mechanism(s) responsible for the unmasking of fetal determinants on the surface of the ABM cell, or for their removal from the FL cell surface, has not been determined. In our system for unmasking, collagenase treatment must precede neuraminidase. After collagenase has acted on its substrate(s), neuraminidase can cleave sialic acid. Whether the cleavage of sialic acid occurs from unmasked glycoprotein, glycolipid or some other macromolecules was not determined. The unmasking may be caused by simple removal of components from the cell surface or could also involve conformational changes at the plasma membrane. An enzymatic function is presumed necessary because heat-inactivated enzymes were ineffective. Other proteolytic enzymes (papain and trypsin) were tested (alone or in combination with neuraminidase) and failed to achieve similar unmasking.

The phenotypic expression of fetal haematopoietic stem-cell determinants on the ABM cell surface after enzymatic unmasking suggests that their transcription is not repressed by the ABM cell. The unmasking of fetal determinants on ABM cells by collagenase and neuraminidase was unique among the tissues studied (adult intestine, liver, spleen and thymus) and enzymes tested. However, it is possible that the expression of these or similar determinants may occur in other conditions, for example, as a function of differentiation or de-differentiation—skin^{10,11}, muscle and lung fibroblasts¹² have all been shown to express fetal determinants when cultured *in vitro*.

Mid-gestation fetal liver cells cross-react immunologically with a variety of viral and chemically induced animal tumours¹³⁻¹⁵, as well as with human malignant melanoma¹⁶, leukaemia^{5,16} and colon carcinoma¹⁷. As transformed cells of various histological types express fetal determinants, it may be physiologically significant that only adult bone marrow cells were demonstrated to carry these determinants in a cryptic fashion.

This work was supported by grants from the American Cancer Society and the US Department of Energy.

Received 2 May; accepted 21 August 1979.

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A virus-free immunogen effective against Epstein-Barr virus

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Epstein-Barr virus (EBV)-associated diseases—infectious mononucleosis (IM), nasopharyngeal carcinoma (NPC) and Burkitt's lymphoma (BL)—constitute considerable world health problems. EBV infection occurs in all human populations and is accompanied by seroconversion and life-long persistence of the virus¹. The antibodies present as a consequence of infection are considered important in controlling the persistent viral infection and in preventing recurrent bouts of IM which is known to be caused by the virus². As EBV infection during childhood does not usually result in illness²⁰, only seronegative adolescents and young adults are susceptible to IM. Similarly, the groups at risk to EBV-associated neoplasms are known. The first group includes children in areas of hyper- and holoendemic malaria in Africa³, where BL is the most common childhood cancer. The other group consists of people, particularly men, of the HLA-2 and Sin 2 haplotypes⁴, of southern Chinese origin amongst whom NPC is the most common tumour⁵. It has been suggested that an anti-EBV vaccine should be developed⁶, and this debate has been stimulated recently by a study further implicating EBV with African BL⁷. I report here the successful production of a DNA-free immunogen which may be readily prepared in large quantities and will successfully invoke a strong neutralising anti-EBV response in experimental animals. This material could form the basis for developing an anti-EBV vaccination programme.

A vaccine against EBV has been considered for several reasons. First, vaccines made from viral antigens have proved highly successful in protecting against tumours caused by Marek's disease herpesvirus in chickens⁸ and by herpesvirus saimiri in marmosets⁹. Second, as stated above, EBV-associated diseases constitute considerable world health problems^{2,3,5} and the populations at risk have been defined. Thirdly, the significance of EBV as a tumorigenic agent in humans could be finally established if it were possible to prevent tumours by vaccination.

Work in this laboratory has been concerned with studying the biochemical nature of the EBV-associated membrane-neutralising antigen complex and using this information to develop an effective anti-EBV vaccine. It was shown previously that a rabbit anti-EBV serum prepared by immunisation with whole purified virus possessed a high neutralising antibody

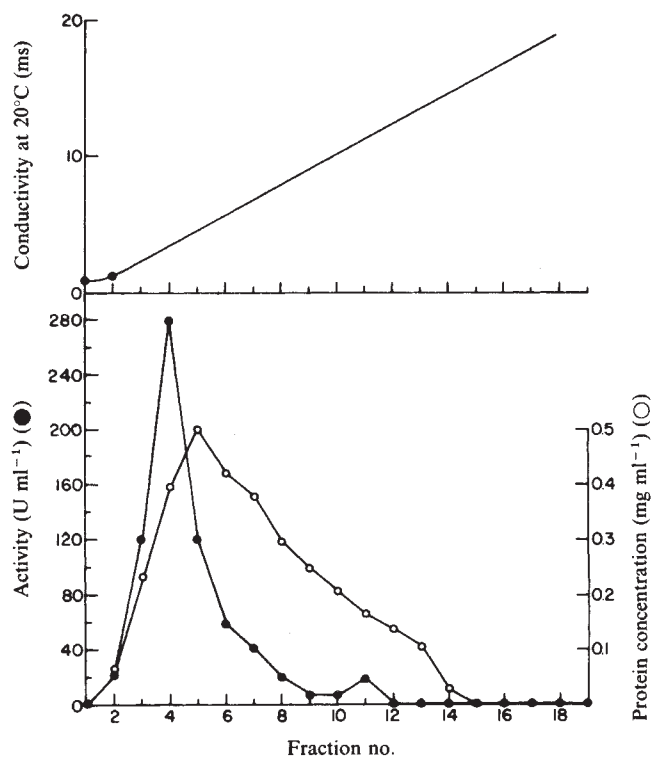


Fig. 1 Fractionation of EBV membrane antigens by DE-52 ion-exchange chromatography. Plasma membranes were prepared essentially as described previously¹⁸ (full details to be published elsewhere). The plasma membranes (20–30 mg of protein from A_{260}/A_{280}) from ~15 g of P3HR-1 cells grown in restrictive conditions were resuspended in 10 mM Tris pH 7.4, 0.25 mM dithiothreitol (DTT) and solubilised by the addition of Triton X-100 (3:1 w/w, Triton X-100:protein) for 30 min at 4°C. The solution was clarified by centrifugation at 200,000 g for 1 h and the supernatant applied to a 4 ml column of DE-52 previously equilibrated with 10 mM Tris pH 7.4, 0.25 mM DTT and 0.2% Triton X-100. The column was washed with 2–3 column volumes and the bound material eluted with a linear gradient (1 ml fractions, 15 ml h⁻¹) of 0–0.4 M potassium phosphate pH 7.4 in the equilibrium buffer. Fractions were all dialysed against phosphate buffered saline (PBS) before assay. Protein (○) was estimated by the method of Lowry¹⁴ and EBV membrane antigens (●) by competition of the ¹²⁵I-PrA assay (see Fig. 2). Approximately 5.52 mg of protein and 2,600 units of membrane antigen were applied to the column.

titre¹⁰. It was not possible to use purified virus as a source for the biochemical characterisation of the antigens recognised by these antibodies because of the technical difficulties involved in concentrating and purifying the small amount of virus released

Table 1 Effect of adding the neutralising (anti-P3HR-1) and control (anti-RAMOS) antisera at various times pre- and post-infection

Time antibody added*	Addition of virus	Infectivity assayed by:			
		DNA synthesis†		Outgrowth‡	
		(³H-thymidine incorporation)		(no. of wells with transformed foci)	
		Anti-P3HR-1	Anti-RAMOS	Anti-P3HR-1	Anti-RAMOS
1 h pre-infection	+	160	4,900	0/6	6/6
2 h post-infection	+	8,100	9,300	6/6	6/6
24 h post-infection	+	7,900	7,200	6/6	6/6
No antibody added	+		7,400		6/6
No antibody added	–		100		0/6

*Antisera were used at a dilution of 1:15.

†For experimental procedure see Fig. 3 legend. Each value was the mean of triplicate experiment, error ≤10%.

‡For experimental procedure see Fig. 4 legend.

by producer cell lines. However, the neutralising antibodies could be specifically absorbed by EBV producer cell lines¹⁰. Therefore, the neutralising antigens are expressed on the plasma membranes of these permanently growing lymphoblastoid cell lines which may be cultured to indefinitely large volumes, thus providing a potential source of large amounts of the antigens. Furthermore, the antigen could be assayed for quantitatively by means of the ¹²⁵I-labelled *Staphylococcus aureus* protein (¹²⁵I-PrA) assay¹⁰ and it has been possible to purify and identify components of the membrane antigen using this assay. The antigen comprises a glycoprotein complex of at least three major (molecular weights (MW) 350,000, 140,000 and 75,000) and several minor polypeptides present on the plasma membrane of all producer cell lines¹¹ and on the virion itself (unpublished results). It was decided, therefore, to try to immunise rabbits specifically against EBV using a partially purified preparation which contained this complex of polypeptides.

Plasma membranes were purified from the P3HR-1 cell line by nitrogen cavitation followed by differential sucrose density centrifugation. The resulting purified plasma membranes contained 4–6% DNA by weight as judged by A_{260}/A_{280} (ref.

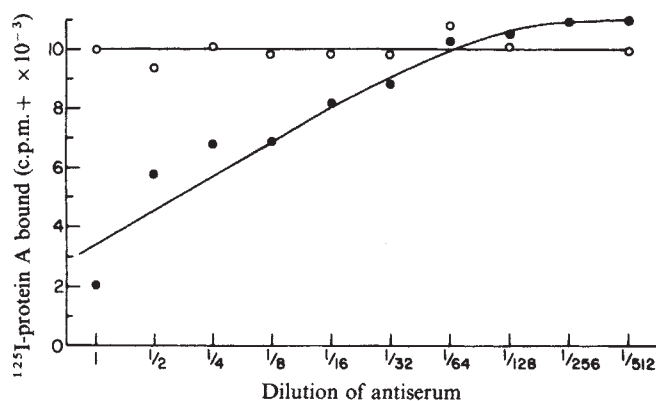


Fig. 2 Assay for the purified (from P3HR-1) and mock-purified (from RAMOS) EBV membrane antigen using competition of the ¹²⁵I-labelled staphylococcal protein A assay. The appropriately diluted antigen (50 μ l) in a final solution of 0.1% Triton X-100 in PBS was incubated overnight with antibody (10 μ l of 1:8 diluted rabbit anti-EBV serum) at 4 °C. The antibody activity in duplicate 25 μ l aliquots was then assayed as described previously¹⁰ except that the P3HR-1 cells used in the assay were first fixed with glutaraldehyde. The activity in units may be calculated as follows: the dilution of antigen required to inhibit the assay by

$$\frac{\text{total volume of sample } (\mu\text{l})}{50}$$

In this experiment an antigen dilution of 1 is equivalent to 160 μ g per ml of membrane protein. A background of 2,600 c.p.m. obtained with normal rabbit serum has been subtracted from these data. ●, Antigens purified from producer cell line P3HR-1; ○, antigens mock-purified from EBV-negative cell line RAMOS.

12). They were solubilised with the non-ionic detergent Triton X-100 and passed over a DE-52 ion exchange column to remove the residual DNA¹³. The material which bound to the column was eluted with a salt gradient. The fractions were assayed for protein by the method of Lowry as modified by Dulley and Grieve¹⁴ and for EBV-specific membrane antigens by inhibition of the ¹²⁵I-PrA binding assay¹¹ (Fig. 1). The tubes with peak activity were pooled, assayed for DNA content¹⁵ and found to have 0.25% DNA by weight compared to Lowry protein (salmon sperm DNA and bovine serum albumin were used as standards).

A mock purification was performed using the EBV-negative lymphoblastoid cell line RAMOS. The pooled fractions from P3HR-1 and RAMOS were then tested for their ability to compete for the rabbit anti-EBV serum in the ¹²⁵I-PrA binding assay (Fig. 2). Only the preparation from P3HR-1 was able to compete, indicating that this material contained the specific EBV membrane antigens.

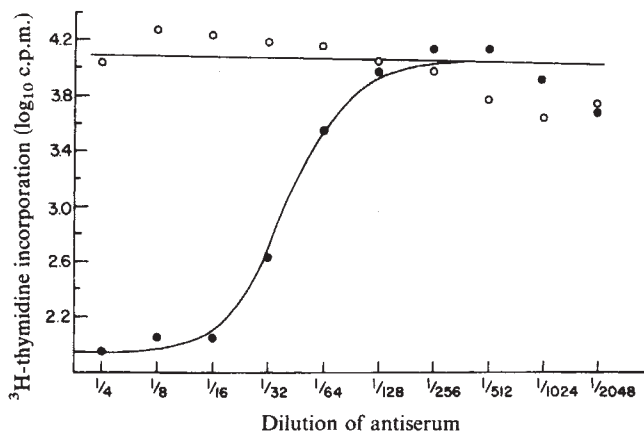


Fig. 3 Neutralisation of EBV infectivity by serum from immunised and mock-immunised rabbits. Infectivity was assayed by measuring the stimulation of DNA synthesis in resting peripheral B lymphocytes. Rabbits were immunised with partially purified antigens from a DE-52 column (Figs 1, 2). The material, consisting of ~100 μ g of protein in 0.5 ml of 0.1% Triton X-100 and PBS, was emulsified in complete Freund's adjuvant and inoculated subcutaneously in the neck and intramuscularly in the thigh. The procedure was repeated three times over the course of two months and the animals were bled 10 days after the last injection. Before use in the neutralisation assay sera were absorbed 4 times for 1 h at room temperature with an equal volume of fresh peripheral lymphocytes to remove nonspecific cytotoxic activity from the sera. The methodology for assaying neutralising activity in sera has been described in detail elsewhere¹⁰. Briefly, the B95-8 strain of virus and sera were incubated for 1 h at 37 °C and then added to fresh B lymphocytes purified from the blood of normal adult humans by immunoabsorbent chromatography. The cells were then incubated for 1 week and proliferation assayed by measuring incorporation of ³H-thymidine. Each experiment was performed in triplicate. ●, Antiserum prepared from a rabbit immunised with partially purified EBV membrane antigens from the EBV producer cell line P3HR-1; ○, antiserum prepared from a rabbit immunised with mock-purified EBV membrane antigens from the EBV-negative cell line RAMOS.

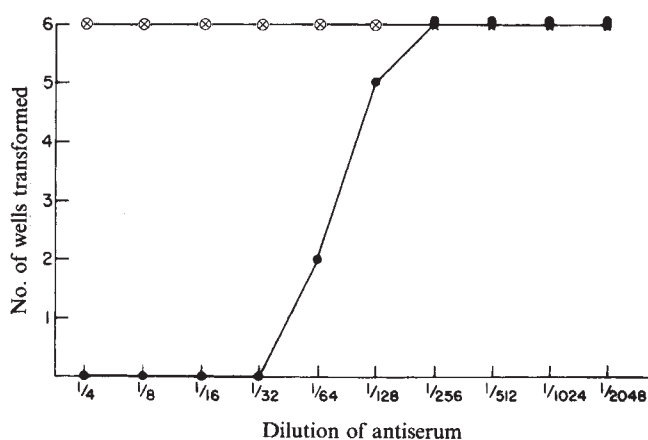


Fig. 4 Neutralisation of EBV infectivity by serum from immunised and mock-immunised rabbits. Infectivity was assayed by measuring the outgrowth of transformed foci. Details as for Fig. 3 legend, except infectivity was assayed by setting up 6 culture wells for each point and counting the number of wells containing transformed foci of proliferating lymphoblasts 20 days postinfection¹⁰. ●, Antiserum prepared from a rabbit immunised with partially purified EBV membrane antigens from the EBV-producer cell line P3HR-1. ○, Antiserum prepared from a rabbit immunised with mock-purified EBV membrane antigen from the EBV-negative cell line RAMOS. x, Normal rabbit serum.

Rabbits were immunised with the partially purified material from RAMOS and P3HR-1 and the resulting antisera were tested for their ability to neutralise the virus *in vitro*. EBV from the B95-8 lymphoblastoid cell line was preincubated with the different sera at various dilutions and the mixture was then used to infect peripheral B lymphocytes from normal adult donors. The infectivity was estimated by measuring either the stimulation of cellular DNA synthesis in the B cells (Fig. 3) or the outgrowth of foci of transformed lymphoblasts¹⁶ (Fig. 4). In both experiments the virus infectivity was neutralised by the antiserum (titre 1:50 to 1:100) raised by immunising with the partially purified EBV membrane antigens from P3HR-1. The equivalent serum raised against mock-purified antigens from the RAMOS cell line was completely ineffective, as was preimmune normal rabbit serum. When the antiserum against P3HR-1 was added a few hours postinfection (Table 1), it was completely ineffective in preventing the infection. These results suggest that the antiserum was specifically neutralising the virus and was not toxic for the infected cells.

These results demonstrate that it is possible to generate a specific EBV-neutralising antibody response in experimental animals with a DNA-free preparation of partially purified membrane antigens from the plasma membranes of EBV-producer cell lines. These results are in agreement with previous observations¹⁹ that EBV membrane antigens removed from super-infected RAJI by papain digestion would invoke an EBV-neutralising response. The production of EBV membrane antigens by super-infection will, however, be difficult to scale up. By comparison, the source of membrane antigens in the present study was EBV producer lymphoblastoid cell lines. As these cell lines are permanently established in culture, they provide a potentially limitless source of the EBV membrane antigens. In

addition, the culture conditions and purification procedures used were simple and will be amenable to scaling up for production of large amounts of vaccine.

As well as the above mentioned associations with neoplasia in man, EBV has been shown to cause tumours in certain New World monkeys, notably marmosets¹⁷. This provides further support for the suggested etiological role of EBV in human carcinogenesis. The next test for the immunogen described here will be to assess its ability to immunise these monkeys against EBV-induced neoplasia.

I thank Dr Clark Edson for advice. This work was supported by NIH grant no. 1 RO1 A11 5310-01.

Received 17 April; accepted 8 August 1979.

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Immunoregulatory effects of a covalent antigen-antibody complex

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It is becoming increasingly clear that any specific immune response must be subject to a control mechanism of the same order of specificity. The basis of such specificity in control almost certainly lies in the antibodies themselves, whether free or cell bound. The response to a given antigen might then be regulated by antibodies specific either for the antigen ('Ab 1') or for the variable regions (idiotypes) of Ab 1. Whereas the latter can interact directly with the binding sites of cellular receptors for antigen, the former can only do so if the antigen is also present to act as a bridge. Furthermore, because an anti-idiotype antiserum is usually prepared against a monoclonal antibody, it will only be able to influence directly those clones of cells which carry the chosen idiotypic, and thus may leave unaffected other clones which are specific for the same antigen but which carry different idiotypes. An immune complex, on the other hand, should influence all clones sensitive to the antigen in question. Experimentally, the injection of antigen-antibody complexes can either increase^{1,2} or decrease^{3,4} the subsequent response to antigen. These effects often require the presence of an intact Fc region⁴ and may differ according to the class (or isotype) of the antibody⁵. The injection of anti-idiotype antisera can have

striking effects on the expression of particular idiotypes, but usually has little effect on the overall response. Again, these effects require the participation of the Fc region of the idiotype antibody⁶, and can vary according to its isotype^{7,8}. To study the regulatory effects of antibody more closely, we have attempted to prepare immune complexes which could influence the overall response, and yet have effects as powerful as those obtained with anti-idiotype antibodies. The immune complexes obtained were of the defined constitution Ag₂Ab, in which stability was ensured by linking antigen to antibody through a covalent bond. We report here that, depending on the conditions used, these complexes induced either priming or tolerance of the response of mice towards subsequent injections of antigen alone. We consider that such complexes could be useful not only as tool for studying immunoregulation, but also as a practical means of influencing immune responses.

The complexes were prepared by mixing hapten-conjugates of the chosen antigen with anti-hapten antibody. Cross-linking was minimised by the use of conjugates which were as far as possible monosubstituted with the hapten. The complexes were rendered stable by the introduction of a covalent bond through the use of the photosensitive affinity label 'NAP' (4-azido-2-nitrophenyl)⁹ as the hapten. As the antigen now becomes effectively an extension of the antibody variable region (Fig. 1), such a complex should mimic an anti-idiotype antibody in its mode of interaction with cellular receptors. The method is summarised in Fig. 1 legend and will be reported in detail elsewhere. As antigen we used pancreatic ribonuclease D (RNase; Sigma).

In the first experiment young adult mice of the hybrid strain (BALB/c × CBA/H)F₁ were each injected intravenously with a single dose of either complex containing 0.16-100 µg of complexed anti-NAP, or free RNase equal to that contained in 4 µg or 100 µg complex, or were left uninjected. Six weeks later the mice were each challenged intraperitoneally with 50 µg of RNase in complete Freund's adjuvant. They were bled 2 weeks

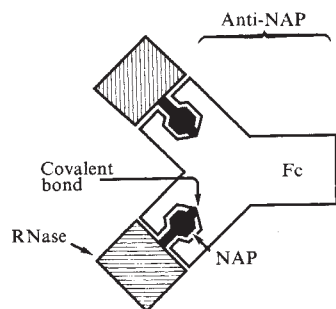


Fig. 1 Diagram of a molecule of covalent complex of NAP-RNase with anti-NAP. NAP was first converted into a form which on the one hand was more reactive with proteins in physiological conditions, and on the other included a spacer chain so as to increase the likelihood of its being sufficiently exposed to interact with the antibody. This was done by reacting NAP with aminocaproic acid in the presence of sodium carbonate, as described by

Fleet *et al.*⁹ for NAP-lysine. The resulting NAP aminocaproic acid (NAP-cap) was coupled to *N*-hydroxysuccinimide to form the succinimide ester (NAPcapOSu) using dicyclohexylcarbodiimide, as described by Becker and Mäkelä¹³ for the analogous DNPCapOSu. The concentration was estimated by optical density after dilution in 0.05 M glycine, pH 8.5, assuming $E_{M}^{485} = 4,800$. An aliquot of NAPcapOSu was stirred rapidly with a 2 molar equivalent of RNase at 10 mg ml⁻¹ in 0.05 M HEPES, pH 7.5, for 1–2 h at room temperature. Uncombined hapten was removed by passage through a Sephadex G-25 column. As RNase has one lysine side chain which is much more reactive than the others at pH values close to neutral¹⁴, it was thought likely that this treatment would result in very few molecules having more than one NAP attached. Anti-NAP antibody was prepared in rabbits by injection of NAPcap-keyhole limpet haemocyanin (9 haptens per 100,000 molecular weight) in complete Freund's adjuvant. It was purified by elution with hapten from an immunoadsorbent column. NAPcap-RNase, prepared with RNase trace labelled with ¹²⁵I, was mixed with anti-NAP antibody in a 2 molar excess of NAP to the antibody-binding sites, and the mixture was illuminated with a standard 60-W bulb for 15 min. The antibody was then concentrated by precipitation with 50% saturated ammonium sulphate, redissolved in a small volume and passed through a gel-permeation column of AcA 34 (LKB). The radioactivity served to monitor RNase, and optical density at 280 nm was mainly due to anti-NAP. It was found that some of the RNase eluted with the anti-NAP whereas the rest followed in the position expected for free RNase. The concentration of RNase in the pooled first peak was obtained from the radioactivity, and multiplied by the ratio of molecular weights to give the concentration of complex, expressed in terms of complexed anti-NAP.

later and their sera titrated for anti-RNase antibody activity by a modification of Farr's ammonium sulphate procedure¹⁰, using ¹²⁵I-labelled RNase at 10⁻⁸M. The results (Fig. 2) show that whereas RNase alone had little or no priming effect, the activity of the complex was extremely strong. It was detectable at the 0.16 µg dose of complex (containing 0.027 µg RNase) and rose to a maximum at the 20 µg dose. For subsequent experiments 10 µg was chosen as the minimal dose required for optimal effect.

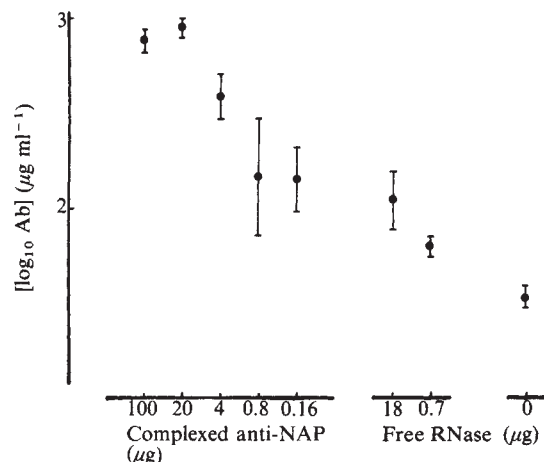


Fig. 2 Dose curve of priming by complex. (BALB/c × CBA/H)F₁ mice were injected intravenously with the doses of RNase complex or free RNase as indicated, 6 weeks before challenge with 50 µg RNase in complete Freund's adjuvant. Sera from a day 14 bleed were assayed by the Farr technique. Each group contains six mice.

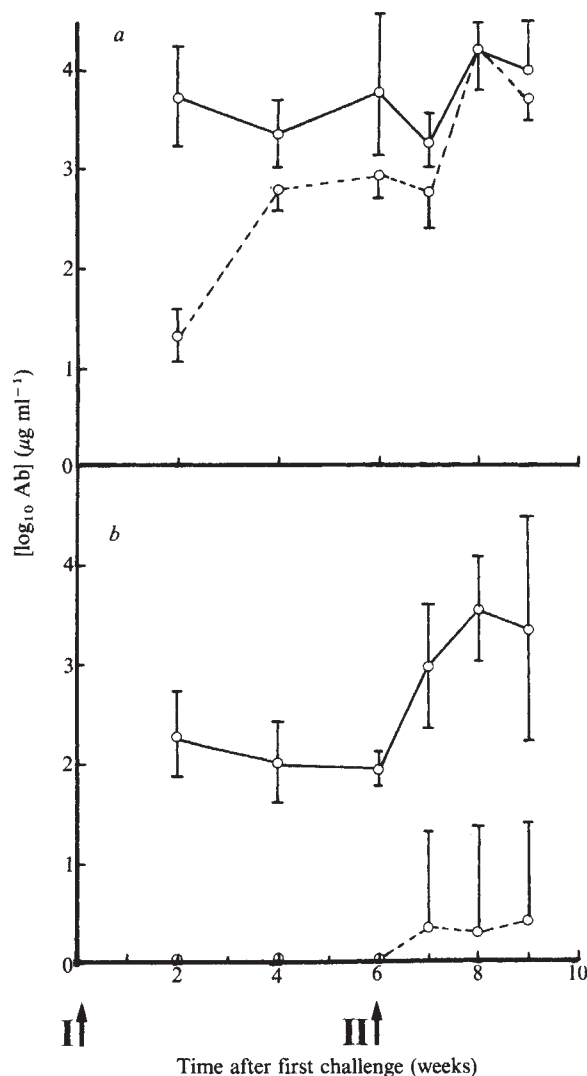


Fig. 3 Challenge with or without adjuvant. Mice were either primed with 10 µg RNase complex (O—O) or left unprimed (O---O). Six weeks later they were challenged with 100 µg RNase (II) either in complete Freund's adjuvant (a) or in saline (b). After a further 6 weeks they received a second challenge (II) of 100 µg RNase in incomplete Freund's adjuvant (a) or saline (b).

The second experiment shows that adjuvant is not necessary to recall the priming induced with covalent immune complex, as this can also be done with soluble antigen. Complex-primed or control mice were challenged with 100 µg RNase in either complete Freund's adjuvant (Fig. 3a) or saline (Fig. 3b). Although unprimed mice made no detectable antibody in response to the first challenge with soluble RNase, complex-primed mice made over 100 µg ml⁻¹. After a second challenge a few of the controls showed a small response, but the primed mice made about 5 mg ml⁻¹ antibody, (over 1,000 times more than controls) and reached about the same level as the maximum achieved by the adjuvant-challenged groups. With adjuvant challenge (Fig. 3a) this maximum titre was achieved by the complex-primed group as early as 14 d after the first challenge, and was not increased further by a second challenge. By contrast, the titres of unprimed mice rose much more slowly, although after the second challenge they did eventually match those of the complex-primed mice.

It seemed possible that this remarkable priming effect was dependent on the rabbit antibody being itself a foreign protein. To test this, some mice were made tolerant to rabbit IgG by

intraperitoneal injection of 1 mg of an ultracentrifuged preparation of the fraction precipitating between 0.81 and 0.92 M sodium sulphate¹¹. One week later they were injected with 10 µg RNase complex as before. Controls received complex alone, equivalent RNase, or no injection. After challenge 6 weeks later with RNase in complete Freund's adjuvant (Fig. 4), the priming effect was again seen in the mice receiving complex alone. By contrast, the mice which had previously been made tolerant to rabbit IgG showed a marked suppression of the response to RNase. Both the priming and suppressive effects were still present, although somewhat diminished, 10 weeks after the first challenge, but were almost abolished after a second challenge.

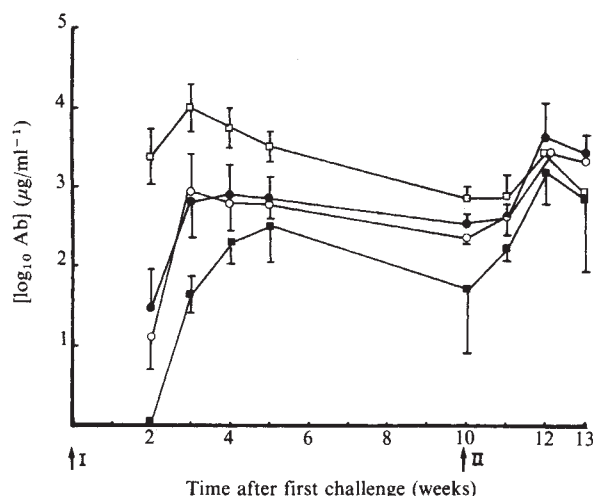


Fig. 4 Effect of prior tolerance to rabbit IgG. One group of mice was injected with 1 mg of rabbit IgG, prepared as described in the text (■), and the other three groups were left uninjected. One week later the four groups received 10 µg RNase complex (□, ■), 2 µg RNase (●), or no injection (○). All groups were challenged twice, at 6 and 16 weeks later, with 100 µg RNase. The first challenge was with complete (I↑) and the second with incomplete (II↑) Freund's adjuvant.

The results described here demonstrate that powerful immunoregulatory effects can be obtained with a covalent antigen-antibody complex. Because rabbit antibody was used in these experiments, it was first expected that the complexes would suppress the response to RNase, by analogy with the suppressive effect of injecting rabbit antibody to a mouse idio-type¹². However, there are several differences between these two situations which may be significant. One of these is the fact that, whereas the complex was prepared from purified antibody, the anti-idiotypic was administered as a whole immunoglobulin fraction, and thus would have contained nonspecific immunoglobulin in considerable excess to the anti-idiotypic antibody. This might have had the same effect as the prior injection of rabbit IgG in our experiment. However, our unpublished experiments using anti-NAPs prepared in various species indicate that the recognition of foreign epitopes on the immunoglobulin is by no means the only factor which determines whether the outcome of injecting such covalent complexes is one of priming or suppression. For example, priming effects have been obtained with complexes formed from an IgG1 fraction of isologous mouse anti-NAP.

We have also studied the mechanism of action of the rabbit anti-NAP-NAP-RNase complex, and found that the Fc portion of the molecule is essential for its immunoregulatory effects, and that covalent bonding greatly enhances these effects, but is not essential for them; these studies will be reported elsewhere. We have obtained equally strong priming effects with several antigens other than RNase, suggesting that this may possibly represent a practical immunisation schedule which could have particular value in that it completely avoids the use of adjuvants.

This work was supported by the National Research Development Corporation and the MRC. C.M. was supported by Fondazione Senatore Pascale, Naples.

Received 22 February; accepted 10 August 1979.

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Graft-versus-leukaemia reactivity induced by alloimmunisation without augmentation of graft-versus-host reactivity

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Supralethal doses of chemoradiotherapy followed by allogeneic bone marrow transplantation are being used to treat patients with acute leukaemia refractory to conventional chemotherapy regimens¹⁻⁵. Despite this approach, leukaemia has recurred in about one-third of the patients¹⁻⁵. One strategy to solve this problem would be to transplant marrow with antileukaemic reactivity to obtain a graft-versus-leukaemia (GvL) effect against residual leukaemia cells⁶. Many workers have demonstrated antileukaemic effects following transplantation of immunocompetent cells from allogeneic donors in animal models, but graft-versus-host (GvH) disease proved to be a complication⁶⁻⁸. Recently, Weiden *et al.*⁹ presented evidence of a GvL effect in human recipients of marrow grafts from HLA compatible allogeneic donors. Unfortunately, this antileukaemic effect was associated with moderate, severe or lethal GvH disease, and the lower probability of recurrent leukaemia was offset by a higher probability of GvH-related mortality⁹. We report here the results that suggest a clinically feasible method of obtaining desired GvL effects while avoiding undesired GvH reactions. Alloimmunisation of CBA (H-2^k) mice was found to induce a population of cells which, when transplanted into leukaemic AKR (H-2^k) mice, led to the destruction of disseminated leukaemia (AKR-L) cells. Furthermore, the transplanted cells caused no augmentation in the mild GvH disease observed when cells from unimmunised CBA donors were administered to lethally irradiated non-leukaemic AKR hosts.

Immunocompetent cells from CBA mice were tested for GvL reactivity in a bioassay system as follows: On day zero, 1×10^5 viable AKR-L cells (obtained by pooling fresh spleen cells from 15 AKR mice bearing spontaneous T cell acute lymphoblastic leukaemia) were inoculated intravenously (i.v.) into lethally

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irradiated (800 rad, total body 4h previous) 8–12-week-old AKR primary hosts. One day later, as the sole antileukaemic treatment, immunocompetent cells (2×10^7 spleen or 10^7 bone marrow plus 10^7 lymph node) from unimmunised, isoimmunised, specifically immunised or alloimmunised CBA donors were transplanted into the AKR primary hosts. The GvL reaction was allowed to proceed for 6 d. To determine whether any viable leukaemia cells remained all spleen cells from each AKR primary host were transferred intraperitoneally (i.p.) to an individual 8–9-week-old AKR secondary recipient on day 7. Using this protocol all but one of 427 control animals, that is, those not given cells from immunised donors, died of leukaemia by day 53. Thus, the proportion of mice alive on day 90 provided a rigorous test of the GvL reactivity of immunised CBA cells.

To evaluate GvH reactivity, 8–12-week-old non-leukaemic AKR mice were exposed to 800 rad total body irradiation and given i.v. injections of immunocompetent cells from unimmunised or immunised CBA donors. The AKR hosts were observed daily for clinical evidence of GvH disease; all deaths were attributed to GvH disease.

The results are shown in Table 1. No antileukaemic effect was observed if AKR primary hosts were given no treatment (group 1) or cells from unimmunised or isoimmunised CBA donors (groups 2 and 3). Cells from specifically immunised CBA donors (group 4) had significantly greater GvL reactivity ($P < 0.001$) than did cells from isoimmunised CBA mice (group 3). Significant GvL reactivity ($P < 0.01$) was induced in CBA donors following immunisation with DBA/1 cells (group 5) in comparison with unprimed CBA cells (group 2); although the difference in GvL reactivity was not significant in comparison with group 3, this may have been due to the small group sizes. Immunisation of CBA donors with lymphoid cells from the other six H-2 incompatible strains of mice (groups 6–11) in each instance induced significant GvL reactivity ($P < 0.001$) in

comparison with group 3, as did either six or two immunisations of CBA donors with pooled lymphoid cells from the seven H-2 incompatible strains (groups 12, 13).

The theoretical and experimental bases for the GvL bioassay model and the rationale for the use of the spleen as the most sensitive bioassay organ have been described previously¹⁰. This bioassay allowed critical evaluation of GvL reactivity without the potentially confounding complications of radiation injury, failure to obtain engraftment and GvH disease. All of these complications, in addition to leukaemia, could have been the cause of death had the primary rather than the secondary recipient been evaluated for survival and autopsied at the time of death.

T lymphocytes appeared to be required for the GvL reaction. Rabbit anti- θ serum and complement treatment of spleen cells from CBA mice alloimmunised with SJL lymphoid cells abolished GvL reactivity (none of 11 mice survived 90 d), whereas treatment of these cells with normal rabbit serum and complement caused no diminution of GvL reactivity (7 of 10 mice survived 90 d). This observation does not exclude a role for natural killer (NK) cells, as it has been suggested that NK cells express low density thy-1 antigen¹¹ and that alloimmunisation augments NK activity¹².

Survival data from GvH assays are presented in Table 1. Cells from specifically immunised CBA mice (group 4) caused a significant increase in GvH-related mortality in comparison with cells from unimmunised (group 2) CBA donors ($P < 0.005$) or isoimmunised (group 3) CBA donors ($P < 0.001$). However, transplanted cells from alloimmunised CBA mice (groups 5–13) caused no significant increase in mortality in comparison with groups 2 or 3.

Bach *et al.*¹³ first proposed pool-priming as a means of generating cytotoxic cells to tumour or virus-infected targets. Zarling *et al.*¹⁴ subsequently demonstrated that lymphocytes sensitised *in vitro* to pooled allogeneic cells were cytotoxic to autologous human leukaemia cells *in vitro*. The findings reported here extend these observations by demonstrating that pool-alloimmunisation *in vivo* generated cells which, when transplanted, killed disseminated AKR-L cells *in vivo*.

Several possibilities exist to explain the effectiveness of alloimmunisation in the induction of GvL reactivity. Activation of T cells which help antigen-specific or nonspecific reactions against the leukaemia targets, activation of NK cells and/or sensitisation of cytotoxic T lymphocytes to alloantigens cross-reactive with leukaemia target antigens are all possibilities. Whatever the mechanism, immunisation of CBA mice with allogeneic H-2-incompatible cells induced GvL reactivity without augmenting the GvH response. To the extent that sensitisation to antigens on cells of a single allogeneic strain in some cases led to relatively weak GvL reactivity, pool-alloimmunisation may circumvent the need to understand which alloantigens are needed to induce the desired GvL effect.

This investigation was supported by NCI grants CA 20484 and CA 18440 and grants from the Leukemia Research Foundation and the Briggs and Stratton Corporation Foundation. Evangeline Reynolds, Eli Godfried, Lisa Lavery, Patricia Matthew and Shirley Stanko provided technical assistance.

Received 8 June; accepted 8 August 1979.

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Table 1 Survival data from assays in which immunocompetent cells from CBA (H-2^k) donor mice were tested for GvL reactivity in leukaemic AKR (H-2^b) mice and for GvH reactivity in nonleukaemic AKR mice

Group	Donor of transplanted cells*	GvL reactivity of donor cells†		GvH reactivity of donor cells	
		No. alive at 90 d/ no. AKR mice transplanted (%)		No. alive at 90 d/ no. AKR mice transplanted (%)	
1	None	1/308 (0)		3/155 (2)	
2	CBA	0/108 (0)		52/77 (68)	
3	CBA α CBA	0/11 (0)		11/12 (92)	
4	CBA α AKR-L _x	36/39 (92)		2/11 (18)	
5	CBA α DBA/1 (H-2 ^a)	2/12 (17)		12/12 (100)	
6	CBA α SJL (H-2 ^b)	24/44 (55)		17/24 (71)	
7	CBA α C57BL/10 (H-2 ^b)	17/23 (74)		12/12 (100)	
8	CBA α B10.P (H-2 ^p)	10/12 (83)		11/12 (92)	
9	CBA α B10.RIII (H-2 ^r)	10/12 (83)		12/12 (100)	
10	CBA α BALB/c (H-2 ^d)	11/12 (92)		12/12 (100)	
11	CBA α A.CA (H-2 ^f)	12/12 (100)		10/12 (83)	
12	CBA α Pool $\times$ 6	12/12 (100)		10/12 (83)	
13	CBA α Pool $\times$ 2	11/12 (92)		11/12 (92)	

Irradiated (800 rad) leukaemic and nonleukaemic AKR hosts received 2×10^7 spleen or 10^7 bone marrow plus 10^7 lymph node cells i.v. from unimmunised (group 2), isoimmunised (group 3), specifically immunised (group 4) or alloimmunised (groups 5–13) CBA donors. χ^2 test was used for significant differences between groups.

* CBA donors received six weekly immunisations i.p. with 1×10^7 lymphoid cells from the indicated strains; the H-2 haplotype of the immunising strain is in parentheses. AKR leukaemia cells (group 4) were irradiated with 3,000 rad. For pool immunisation (groups 12 and 13), 1×10^7 lymphoid cells from each of the seven H-2-incompatible strains were combined and injected i.p.; mice in group 13 received only two immunisations. CBA mice were used as donors one week after the last immunisation.

† A bioassay system was used to measure the GvL reactivity of CBA donor cells. All deaths were due to leukaemia.

Complement-fixing IgG1 constitutes a new subclass of mouse IgG

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Four IgG isotypes (IgG1, IgG2a, IgG2b, IgG3) have been distinguished in the mouse¹⁻³. The similarity of the physico-chemical properties of the different isotypes has to a large extent prevented their purification from serum and this has complicated functional studies on IgG antibodies in sera from infected or immunised mice^{1,2,4}. The availability of immunoglobulin-secreting plasmacytomas⁵, however, has made the production of isotype-specific antisera possible; the use of these antisera has shown immunoglobulin of each isotype to exist in sera from normal animals^{3,5,6}. IgG1 antibodies, partially separated from IgG2 by electrophoresis in agar, have been reported² to be incapable of sensitising sheep red blood cells (SRBC), passively coated with antigen, for lysis by complement (C) although the antibodies agglutinated the cells and also mediated passive cutaneous anaphylactic (PCA) reactions in the mouse. The IgG2 antibodies, which mediated PCA reactions in the guinea pig but not in the mouse, lysed the antigen-coated SRBC in the presence of mouse C. It seems on the basis of this single report that mouse IgG1 has been widely accepted⁶⁻⁸ to be a 'non-C-fixing' class of immunoglobulin—one which, on association with antigen, is unable to activate C. We have now purified IgG1 from the serum of mice immunised with SRBC and in this report present evidence that the IgG1 antibodies are not only haemolytic in the presence of C, but that they apparently account for more than half of the SRBC-specific, C-fixing IgG antibodies in the serum of the immunised animals.

Six-week-old female LACA mice from a random-bred specific-pathogen-free colony were conventionalised for 3 weeks before being injected intravenously with 1×10^8 SRBC in 0.2 ml of saline on days 0 and 21. Blood was collected on day 32, pooled and the serum stored at -20°C . IgG1, IgG2a and IgG2b immunoglobulin was purified from the serum by fractionation on protein A-Sepharose CL-4B (Pharmacia) as previously described⁹. The peak eluate fractions were pooled, dialysed against 0.15 M NaCl–15 mM NaN_3 and stored at 4°C to minimise aggregation.

Each IgG preparation was titrated for SRBC-specific antibodies by haemagglutination as well as by C-dependent haemolysis. The haemolytic titrations (Fig. 1) showed that each

preparation contained antibodies which were lytic for SRBC in the presence of guinea pig C. This activity was SRBC specific, since neither agglutination nor lysis was observed when human or guinea pig RBC were used as target cells and the activity could be removed by adsorption to SRBC but not to the other types of cells (data not shown). Because IgG1 antibodies were believed to be incapable of activating C (ref. 2), it was thought possible that the IgG1 preparation contained trace amounts of SRBC-specific IgM antibody or aggregated IgG1 which might bind to the cells and activate C nonspecifically, perhaps via the alternative pathway. Thus, all of the IgG1 immunoglobulin was

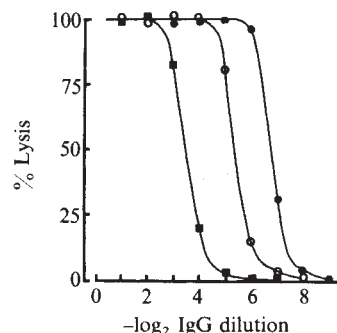


Fig. 1 Titration of IgG1, IgG2a and IgG2b for capacity to lyse SRBC in the presence of guinea pig C. All reagents were diluted in Veronal-buffered saline (pH 7.4) containing 0.15 mM CaCl_2 and 0.5 mM MgCl_2 (VB^{2+})¹⁸, supplemented with 0.2 mg ml^{-1} bovine serum albumin and 7.5 mM NaN_3 (VB^{2+} - BSA). Fresh guinea pig serum, absorbed with SRBC (3 ml packed cells per 30 ml serum; 30 min, 0°C), was stored at -20°C in 0.25-ml aliquots which were thawed as required at room temperature and used immediately. Two-fold serial dilutions of antibody (0.2 ml) were mixed with 0.4 ml of 0.5% SRBC (3×10^7 cells) with rapid mixing. After incubation at 37°C for 30 min, 0.2 ml of a 1:20 dilution of guinea pig serum was added. The tubes were incubated for 60 min at 37°C , immediately centrifuged and the absorbance at 541 nm determined for each supernatant. Lysis is expressed relative to water-lysed (100%) and SRBC+C (0%) controls. No lysis of antibody-sensitized SRBC was observed in the absence of C. ●, IgG1; ○, IgG2a; ■, IgG2b.

chromatographed on Sephadex G-200 to separate IgM and aggregated IgG from unaggregated (monomeric) IgG molecules. As depicted in Fig. 2, this fractionation yielded a single, homogeneous peak of monomeric IgG1 which represented, within the limits of detection, all the protein applied to the column. All the detectable haemolytic and haemagglutinating antibody activity was associated with this monomeric IgG1 protein peak. The fractions representing the void volume, where IgM and aggregated IgG would be expected to elute, contained neither agglutinating nor haemolytic activity. The IgG1 haemolytic activity was therefore not due to 19S IgM antibody, to aggregated IgG1, or to monomeric (8S) IgM (an unlikely possibility) since the latter would have eluted with the leading edge of the IgG peak (the same column has been used successfully to resolve IgE and IgG1 antibodies, measured by PCA reactions¹⁰). Subsequent chromatography of samples of the IgG2a and (separately) the IgG2b indicated that these preparations similarly consisted of only monomeric IgG.

To determine the purity of the IgG preparations, each was analysed by radioimmunoassay for immunoglobulin content. The IgG1 used for this analysis and for subsequent experiments was recovered from fractions 38–40 of the Sephadex G-200 fractionation (Fig. 2), corresponding to the top of the eluant IgG1 peak. The results of the analyses (Table 1 legend) showed that the IgG2a and IgG2b contained only minor amounts of other immunoglobulins totalling 0.94% and 6.8% by weight respectively. No contaminating immunoglobulin could be

Table 1 SRBC-specific antibody activity of IgG preparations

IgG*	Haemagglutination†	Haemolysis‡
IgG1	1/174	1/82.8 (1/146)
IgG2a	1/36	1/73.0 (1/93.6)
IgG2b	1/<1	1/12.8 (1/70.7)

* Analysis (μg per ml; protein, IgG1, IgG2a, IgG2b, IgM). IgG1: 568, 502, <1, <0.4, <0.08; IgG2a: 780, 5.7, 514, 1.24, 0.38; IgG2b: 181, 3.5, 8.08, 210, 0.79; Unfractionated serum: -, 2,580, 2,770, 930, 333. Protein concentrations estimated from A_{280} ($E_{1\%}^{1\text{cm}} = 14$), immunoglobulin levels by radioimmunoassay^{9,16}.

† Twofold serial dilutions of antibody (0.2 ml) in VB^{2+} -BSA (see Fig. 1 legend) mixed with SRBC (0.2 ml, 1%). Endpoint chosen as the maximum dilution causing agglutination, taken to a fraction (1/4, 1/2, 3/4, 1) ($\log_2$) of last positive well.

‡ Dilution of antibody causing 50% lysis using 1:20 C. Endpoints were determined by regression analysis of von Krogh plots¹⁸. Values in parentheses are the specific haemolytic titres calculated for a 1 mg ml^{-1} solution of each IgG.

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detected in the IgG1 preparation (IgM, IgG2a and IgG2b, together <0.26% by weight). The analyses corresponded closely to the protein concentrations, indicating little or no contamination by other serum proteins. This was confirmed by electrophoresis of each IgG (labelled with ^{125}I) on SDS-polyacrylamide gels¹¹ (not shown). In non-reducing conditions, the radioactivity migrated as a single band with a mobility characteristic of intact IgG. After reduction, each sample yielded only two peaks corresponding in mobility to heavy (γ) and light (L) chains. The L chain peak contained 24–28% of the total radioactivity (theoretically, 30%).

The SRBC-specific antibody titres of the IgG preparations are shown in Table 1. Most of the antibody, haemagglutinating and haemolytic, was associated with the IgG1. The specific haemolytic activities, shown in Table 1 in parentheses, indicated that on a weight basis the IgG1 possessed 1.56 and 2.06 times more haemolytic antibody than the IgG2a and IgG2b respectively. The lytic activity associated with the IgG1 could not therefore have been due to contaminating IgG2a or IgG2b, both of which were undetectable. From the concentrations of IgG1, IgG2a and IgG2b in the original serum pool (measured by radioimmunoassay; Table 1 legend) and the calculated specific haemolytic activity of each of the three IgG isotypes, it can be estimated that of the total SRBC-specific haemolytic IgG antibody present in the serum, 54% was due to IgG1, 37% to IgG2a and 9% to IgG2b. The validity of this calculation is conditional on the specific haemolytic activities of the IgG isotypes being unaltered by the purification procedure. The possibility of partial denaturation in the conditions used for elution from the protein A-Sepharose (IgG1, pH 5.8; IgG2a, pH 4.5; IgG2b, pH 3.5) cannot be excluded, although the fractionation was carried out at 4 °C and the fractions were neutralised as they were collected. The observed haemolytic activity of the IgG fraction of the mouse antiserum (isolated by passage of the serum through Sephadex G-200) could, however, be fully accounted for by its content of each IgG isotype (determined by radioimmunoassay) using the specific haemolytic activities calculated above.

As the IgG1 was unaggregated, activation of C by the alternative pathway seemed unlikely. To assess whether the observed haemolysis resulted from activation of C by the classical pathway, the effect of EDTA or EGTA on lysis by each type of IgG was determined. EGTA blocks classical pathway activation but has little effect on the alternative pathway, whereas EDTA inhibits both routes¹². The results (Table 2) demonstrate clearly that haemolysis by each IgG isotype was mediated entirely by classical pathway activation. Similar results, including those for IgG1, were obtained when normal mouse serum rather than guinea pig serum was used as the source of C.

These findings are apparently in conflict with present opinion^{2,6–8} that mouse IgG1 is a 'non-C-fixing' class of immunoglobulin. In other experiments¹³, however, we have isolated IgG1 from the sera of mice immunised with several

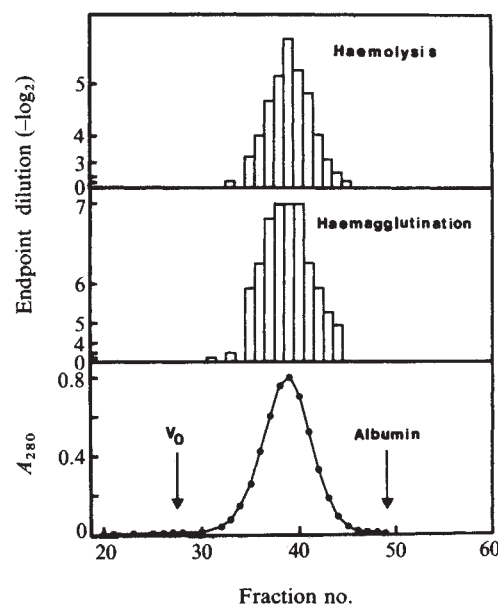


Fig. 2 Sephadex G-200 chromatography of purified IgG1 immunoglobulin. Column size: 2.2 × 90 cm; sample: 4.1 ml (14.4 mg IgG1); eluant: phosphate-buffered saline (pH 7.4) containing 7.5 mM $\text{Na}_2\text{S}_2\text{O}_5$; flow rate: 15.3 ml h^{-1} . Each fraction (4.1 ml) was assayed for protein (A_{280}) and for SRBC haemagglutinating and haemolytic antibody. A trace of ^{125}I -labelled BSA was added to the sample as an intrinsic marker. The elution positions of albumin and of excluded macromolecules (V_0), determined separately using Blue Dextran 2000 (Pharmacia), are indicated.

different antigens and have shown that, in certain instances, IgG1 antibodies are unable to fix C and can effectively block lysis of target cells by C-fixing antibodies through competition for antigenic sites on the target cell surface. Since both types of IgG1 antibody can be produced by inbred strains of mice¹⁴, the genes coding for each type of γ chain cannot be allelic. The C-fixing IgG1 therefore seems to represent a previously undescribed mouse immunoglobulin isotype. We propose that the 'non-C-fixing' and C-fixing IgG1 be termed IgG1a and IgG1b respectively, according to the nomenclature for mouse IgG isotypes based on Fahey *et al.*^{1,6}. Whether these functionally distinct types of IgG1 correspond to the two antigenically distinct IgG1 myeloma proteins described by Stanislawski and Mitard¹⁵ remains to be shown.

We thank Mr G. Ekserdjian for technical assistance and Mrs C. Heanes for typing the manuscript. This work was supported by a grant from the National Health and Medical Research Council of Australia.

Received 4 June; accepted 13 August 1979.

Table 2 Haemolytic activity of IgG antibodies in the presence of EGTA or EDTA (% lysis)

Sensitising agent	Control	EGTA	EDTA
IgG1	96.0	2.7	3.7
IgG2a	95.1	3.1	3.5
IgG2b	94.5	4.1	3.3
Inulin	65.2	48.0	2.9

Each tube contained four 50% lytic units of IgG or 5 mg of inulin (Merck). Inulin activates C by the alternative pathway, mediating 'bystander cell lysis' (ref. 17), and was included as a control for alternative pathway activation. The assay diluent was VB²⁺-BSA with EGTA or EDTA (final concentration, 10 mM) added as indicated. The assay was as described in Fig. 1 except 0.2 ml of guinea pig serum (diluted 1:2 in the appropriate diluent) was used. The values shown are the average of triplicate tubes.

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Removal of ambient K⁺ inhibits net Na⁺ transport in toad bladder by reducing Na⁺ permeability of the luminal border

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Sodium crosses epithelia in two steps: an influx into the cell through the luminal membrane down an electrochemical gradient, and an energy-requiring efflux across the serosal border against a gradient. Koefoed-Johnsen and Ussing¹ originally proposed that cellular efflux of Na⁺ at the serosal border of frog skin is coupled to K⁺ influx by an exchange pump. It was, therefore, assumed that the inhibition of active Na⁺ transport following the removal of K⁺ from the serosal side was due to a reduced pool of K⁺ available for the coupled exchange. Indirect evidence suggests that this is not correct. (1) K⁺ influx from the serosa does not correlate to any significant degree with active Na⁺ transport²⁻⁵. (2) Removal of Na⁺ as well as K⁺ from the serosal medium substantially reduces the inhibitory effect of K⁺ removal on active transport^{2,6}. (3) One would predict that if K⁺ removal did inhibit active transport only at the serosal pump site, then as active transport decreased the 'active transport pool', that is, the mucosally derived cell Na⁺, would increase as it does with ouabain. Studies by Robinson and Macknight⁷, however, showed that the mucosally derived pool does not change on removal of external K⁺. Essig and Leaf² suggested that removal of serosal K⁺ inhibited Na⁺ transport by decreasing the permeability of the luminal membrane. They showed that when K⁺ was removed from the serosa, there was a decrease in the amount of ²²Na taken up into the tissue after exposure of the luminal surface to the isotope for 5 min. We have recently found that tissue uptake of ²²Na after exposure of toad bladders to the isotope is linear with time for only up to 24 s. Because only the linear portion of the uptake is a measure of the Na⁺ flux across the luminal border⁸, it is clear that longer periods of uptake are more representative of the steady-state isotope distribution in the tissue. This value is controlled not only by the luminal permeability but also by the serosal permeability and the rate of net transport. These issues prompted us to re-investigate the effect of low external K⁺ on Na⁺ movement across the luminal border. We report here that the Na⁺ transport inhibition seems to be due to a reduced Na⁺ permeability at the luminal border.

Toad bladders were exposed to ²²Na on the mucosal side for 15 s using Thompson and Dawson's modification⁹ of the method of Biber and Curran¹⁰. The uptake, corrected for extracellular contamination (we shall call this the influx) is determined by two factors, the permeability of the luminal border to Na⁺ and the electrical driving force across that membrane.

The Na⁺ influx was identical to the simultaneously measured rate of net Na⁺ transport evaluated as the short circuit current (Table 1). That the influx is actually a measure of Na⁺ movement across the luminal border rather than a measure of Na⁺ transport across the whole tissue is seen in experiments using amiloride and ouabain. Although these agents reduced net Na⁺ transport equally they affected the influx differently. Amiloride, acting on the mucosal side, abolished transport and influx. Ouabain, however, inhibited net Na⁺ transport by 90% but reduced influx by only 40%.

When K⁺ was removed from the serosal side the short-circuit current declined to 50% of its initial value in 20 min. Measurement of the influx at that time revealed that it was reduced by an equivalent amount (Table 1 and Fig. 1). As the influx is the product of the Na⁺ permeability of the apical membrane and the

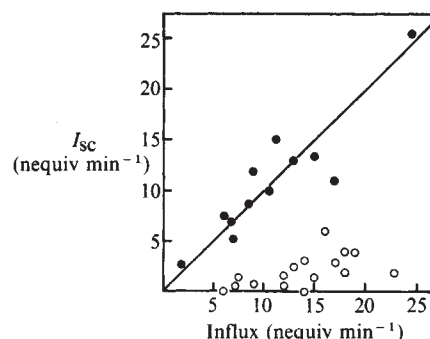


Fig. 1 Plot of the simultaneously measured short circuit current (I_{sc}) with respect to the influx (J) after 20 min of either ouabain (1.0 mM, ○) or K⁺-free (●) inhibition. The solid line represents the identity between the short circuit current and the influx obtained in control bladders. The line describing the K⁺-free points is $I_{sc} = 0.86 (\pm 0.14)J + 1.5 \text{ nequiv. min}^{-1}$ ($r = 0.89$) and that of the ouabain points is $I_{sc} = 0.22 (\pm 0.08)J - 0.79 \text{ nequiv. min}^{-1}$ ($r = 0.60$). Mucosal Na⁺ concentration was 12 mM in all experiments.

electrical driving force across that membrane, the cause of the decrease could be due to either factor. Recent studies by deLong and Civan⁵, however, have shown that removal of K⁺ had no effect on the cell potential in toad bladder. Further, as can be seen in Fig. 1, the influx, in some instances, was reduced to near-zero levels, suggesting that if the only cause for the reduction was a change in the potential, the intracellular voltage must have attained a very large positive value. These considerations suggest that the permeability of the luminal border decreased to cause a reduction in the influx and consequently the short-circuit current.

The identity between influx and short circuit current indicates that the reduction in net transport is due to a reduced Na⁺ entry

Table 1 Effect of ouabain and K⁺-free serosal solution on short circuit current and influx

Serosal solution changed to	Initial I_{sc} (nequiv. min ⁻¹)	Following serosal change	
		I_{sc}	Influx
Ringer's (control) ($n = 9$)	19.5 ± 2.1	21.3 ± 2.8	20.6 ± 3.1
Ouabain (1.0 mM) ($n = 16$)	24.0 ± 1.6	2.2 ± 0.4	14.0 ± 1.2
K ⁺ -Free ($n = 12$)	22.9 ± 1.6	11.0 ± 1.7	10.9 ± 1.8
Amiloride			
Ringer's ($n = 4$)	21.1 ± 1.9	0	0.1 ± 0.1
Ouabain ($n = 4$)	20.4 ± 0.9	0	-0.2 ± 0.3
K ⁺ -Free ($n = 3$)	19.1 ± 7.9	0	0.1 ± 0.2

In the above experiments small pieces of toad bladder (1.5 cm²) were mounted in modified Ussing chambers and short-circuited until the current reached a steady value (initial I_{sc}). For all experiments the mucosal solution consisted of (in mM) NaCl 10, NaHCO₃ 2.5, KCl 3.5, CaCl₂ 1.0 and choline chloride 100. The serosal buffer contained NaCl 110, NaHCO₃ 2.5, KCl 3.5 and CaCl₂ 1.0. The KCl was omitted without substitution in the K⁺-free studies. After the I_{sc} had reached a steady level the serosal solutions were changed. Twenty minutes later the I_{sc} was recorded while the ²²Na uptake was being studied. For the control bladders the slope of the influx as a function of short circuit current was 0.95 ± 0.24 , intercept $0.5 \pm 5.6 \text{ nequiv. min}^{-1}$, $r = 0.83$. In a larger series of control bladders ($n = 20$), influx = $0.97 (\pm 0.14) + 0.03 (\pm 1.10) \text{ nequiv. min}^{-1}$, $r = 0.86$. In a separate experiment amiloride (0.1 mM) was added to the mucosa 20 min after the same serosal changes made in the previous experiment.

into the cell. However, these results do not rule out other effects of K^+ -free media on the Na^+ pump. Figure 2 shows that the inhibition of the luminal permeability is the major cause of the reduction in net transport. Amphotericin B, when added to the mucosal medium, induces channels in the luminal membrane which accelerate Na^+ entry into the cell¹¹. When removal of external K^+ reduced the short circuit current to 30% of its initial value, mucosal addition of $15 \mu g ml^{-1}$ of amphotericin B increased the short circuit current, restoring it to near the initial rate. Ouabain completely inhibited this new current, indicating the current was due to the activity of the Na^+ pump. These results show that the inhibition of net transport by removal of external K^+ was due largely to the reduction of the luminal permeability to Na^+ .

As the Na^+ pump is probably the $(Na^+ + K^+)ATPase$, it is rather puzzling that amphotericin enhanced net transport in the absence of external K^+ . The most probable explanation is that, although there is no K^+ in the bulk solution, it is unlikely that the K^+ concentration in the unstirred layer next to the serosal border is actually zero. Hence, the pump may still be operating in the Na^+, K^+ mode. Alternatively, it may be exchanging Na^+ for Na^+ in an 'electrogenic' manner.

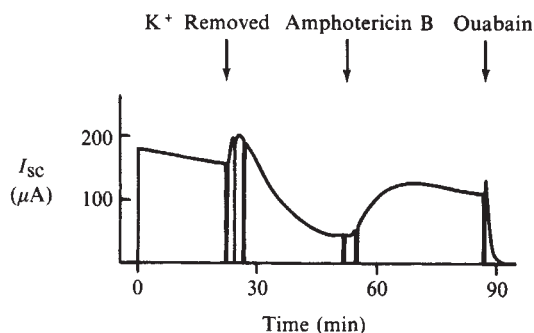


Fig. 2 The tracing obtained from a toad hemibladder mounted in a standard Ussing chamber. As marked, after an initial period during which the bladder reached a steady value, the serosal K^+ was removed (the solution was changed three times). After 20 min, when the current had fallen to ~30% of the initial value, amphotericin B ($15 \mu g ml^{-1}$) was added to the mucosal side. Thirty minutes later ouabain (1 mM) was added to the serosal bath in the presence of mucosal amphotericin. The average values of the seven experiments carried out were (in μA): initial I_{sc} , 175 ± 24 ; 20 min after serosal K^+ removal, 68 ± 13 ; peak response to amphotericin, 129 ± 19 ; after ouabain, 0. The mucosal Na^+ concentration was 12 mM.

When serosal K^+ is removed the cells lose K^+ and gain Na^+ concomitantly with the decline in Na^+ transport^{2,7}. Removal of serosal Na^+ and K^+ prevents the changes in cell ionic composition and the fall in active transport. These data suggest that changes in intracellular ionic composition are the cause of the reduced permeability of the luminal membrane. The increase in cell Na^+ concentration could reduce the permeability of the Na^+ channels by an allosteric mechanism similar to that invoked by Lindemann¹² and Cuthbert¹³ to explain the effect of external Na^+ concentration on the Na^+ permeability of the luminal membrane. Alternatively, an increase in cell Na^+ might increase cytosolic Ca^{2+} (ref. 14) through $Na^+ : Ca^{2+}$ exchange across cell and mitochondrial membranes. As with Na^+ , external Ca^{2+} has been shown to reduce the Na^+ permeability of the apical membrane¹⁵. Whether any of these or other mechanisms are involved must be determined by future studies.

Received 15 June; accepted 10 August 1979.

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Direct addition of insulin inhibits a high affinity Ca^{2+} -ATPase in isolated adipocyte plasma membranes

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The mechanism by which insulin regulates cellular metabolism remains unknown although indirect evidence suggests that alterations in intracellular calcium are important. More specifically, it has been proposed that insulin triggers an increase in intracellular calcium which is responsible for the subsequent modification of metabolic activities^{1,2}. The cell maintains a large electrochemical gradient for ionised calcium between the cytoplasm ($<10^{-6}$ M, as determined for muscle³ and nerve⁴) and the extracellular environment ($>10^{-3}$ M). The plasma membrane may, therefore, be important in the regulation of calcium homeostasis, as a slight alteration in the processes maintaining this gradient could result in marked changes in cytoplasmic calcium. One such process is the active extrusion of calcium from the cell by a high affinity calcium-stimulated ATPase (Ca^{2+} -ATPase). Such a mechanism has been well established in red cells⁵ and is postulated in nerve⁶, liver⁷ and muscle⁸. We have identified a high affinity Ca^{2+} -ATPase in a plasma membrane-enriched subcellular fraction isolated from rat adipocytes⁹ which may provide the enzymatic basis for a calcium extrusion pump. We demonstrate here that the Ca^{2+} -ATPase is specifically inhibited by the direct addition of physiological concentrations of insulin to the isolated plasma membranes. This effect suggests that direct regulation of calcium homeostasis may represent an important event in the mechanism of action of insulin.

The high affinity Ca^{2+} -ATPase of rat adipocyte plasma membranes had an apparent half saturation constant ($K_{0.5}$) of $0.14 \pm 0.04 \mu M$ (mean $\pm$ s.e.m. of eight experiments as in Fig. 1) for ionised calcium. A Hill plot of the data in Fig. 1 yielded a straight line with a Hill number of 1.5 (Fig. 1 inset). The maximal activity of the high affinity Ca^{2+} -ATPase was 196 ± 41 nmol ^{32}P mg^{-1} min^{-1} (mean $\pm$ s.e.m., five experiments) in membranes stored for less than two days. Activity decreased markedly after longer storage. Another saturable component appeared at higher calcium concentrations ($K_{0.5} \approx 74 \mu M$), indicating the presence of a low affinity site similar to that previously reported for this tissue¹⁰. The high affinity enzyme yielded a biphasic response to varying ATP concentrations, also representing the sum of two saturable components, one with $K_{0.5} \approx 0.8 \mu M$, the other with $K_{0.5} \approx 27 \mu M$ (data not shown). The presence of 20 mM sodium, 20 mM potassium or 0.5 mM ouabain did not affect the Ca^{2+} -ATPase, but activity was completely inhibited by 0.2 mM lanthanum. The enzyme had an absolute requirement for the endogenous magnesium that was present in the plasma membrane preparation. Chelation of magnesium by *trans*-cyclohexane-1,2-diamine-*N, N, N', N'*-tetraacetic acid

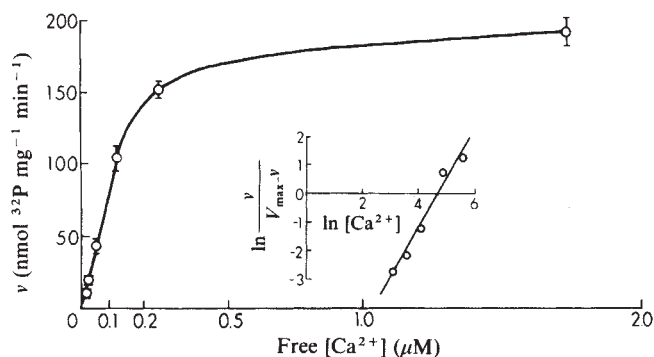


Fig. 1 Calcium dependence of the high affinity Ca^{2+} -ATPase in adipocyte plasma membranes. Isolated adipocytes were prepared from the epididymal fat pads of 120 g Sprague-Dawley rats³⁵ and the plasma membrane fraction obtained by a modification of the method of McKeel and Jarrett¹¹ in which EDTA was omitted from the fractionation and homogenisation media³⁶. The membrane fraction used in these studies has been previously characterised and has been shown to be highly enriched in plasma membranes by the use of the specific marker enzymes adenylate cyclase and 5'-nucleotidase³⁷. The distribution of the high affinity Ca^{2+} -ATPase was shown to parallel the distribution of 5'-nucleotidase among the subcellular fractions (unpublished observations). These plasma membranes, which specifically bind ^{125}I -insulin³⁸, were either used immediately or within two days after storage at -70°C . Ca^{2+} -ATPase activity was quantitated by monitoring ^{32}P released from γ - ^{32}P -ATP and is expressed as nmol ^{32}P per mg protein per min³⁹. Protein values were obtained by a previously described method⁴⁰. The assay medium contained 20 μg protein ml^{-1} with 200 μM EGTA, 100–200 μM CaCl_2 (equivalent to 0.02–1.80 μM free Ca^{2+}), 1 mM Na_2ATP , 20 mM Na azide and 12.5 mM Tris-PIPES, pH 7.5 at 37°C . Endogenous calcium in the assay medium was determined to be 4.5–5.0 μM by analysing the stock reagent solutions used by atomic absorption spectroscopy⁴¹. The total calcium concentrations used to calculate the ionised component take into account the endogenous calcium present in the reagents. Ca^{2+} -ATPase activity was determined by subtracting values obtained with EGTA alone from those obtained with calcium plus EGTA. Each point represents the mean $\pm$ s.e.m. of quadruplicate determinations. Inset: Hill plot of the data. Free $[\text{Ca}^{2+}]$ was calculated using a Ca-EGTA apparent association constant of $10^{7.832} \text{ M}^{-1}$ which was determined for pH 7.5 as described by Portzehl *et al.*³. The concentration for ionised calcium was corrected for any effect of ATP using the equations described by Portzehl *et al.*³ and Schatzmann⁴² and a Ca-ATP apparent association constant of $10^{3.930} \text{ M}^{-1}$, calculated at pH 7.5. All cation-ligand association constants were obtained from Sillen and Martell⁴³.

(CDTA), which has a high affinity for magnesium, totally abolished Ca^{2+} -ATPase activity. Activity could be fully restored by the addition of micromolar concentrations of free magnesium.

The high affinity Ca^{2+} -ATPase was located predominantly in the plasma membranes. This was determined by assessing its relative distribution among subcellular membrane fractions. The specific Ca^{2+} -ATPase activities in mitochondrial and microsomal (enriched in endoplasmic reticulum) fractions were 10 and 32%, respectively, of that of the plasma membrane fraction and could be accounted for by plasma membrane contamination^{11,12}.

Direct addition of insulin to the plasma membranes resulted in a highly significant decrease in Ca^{2+} -ATPase activity at various calcium concentrations in the region of the $K_{0.5}$ for calcium (Table 1). The per cent inhibition by insulin was greatest at the lowest calcium concentration and became less at higher concentrations, suggesting that insulin interaction with the plasma membranes may result primarily in a decreased affinity of the enzyme for calcium. The specificity of the insulin effect was shown in two ways. First, the inhibitory effect was concentration dependent over the physiological range for insulin (Fig.

2), increasing sharply between 0 and 50 μU insulin ml^{-1} and plateauing between 50 and 100 μU ml^{-1} . This concentration dependence is similar to other physiological responses to insulin such as glucose transport and oxidation¹³. Second, direct addition of equimolar concentrations of either desoctapeptide insulin, an analogue which has ~1% of the biological activity of insulin, or insulin which had been inactivated by boiling for 15 min did not inhibit the Ca^{2+} -ATPase. A similar inhibition was observed in plasma membranes prepared from cells which were pretreated with 100 μU insulin ml^{-1} at 37°C for 15 min compared to plasma membranes simultaneously prepared from control cells¹⁴ (data not shown). As the same effect is also seen in the intact cell, this similarity suggests that inhibition of the Ca^{2+} -ATPase by the direct addition of insulin to the plasma membranes is not an artefact.

The characteristics of the high affinity Ca^{2+} -ATPase in adipocyte plasma membranes strongly suggest that it may provide the enzymatic basis for a pump which extrudes calcium from the cell analogous to the well-characterised calcium transport enzyme in red cell plasma membranes⁵. Both enzymes are similar in that they: (1) require magnesium, (2) possess a $K_{0.5}$ for calcium close to the concentration of ionised calcium in the cytoplasm, (3) have similar low and high affinities for ATP, (4) are relatively independent of sodium or potassium ions, (5) are insensitive to ouabain, (6) are completely inhibited by lanthanum, and (7) possess a Hill number greater than one and less than two^{5,15,16}. It is not surprising that such a calcium extrusion pump may exist in adipocytes as its presence has previously been suggested from calcium flux studies using intact cells¹⁷.

An insulin-induced inhibition of calcium extrusion via inhibition of a high affinity pump enzyme could be a critical event in the mechanism of insulin action. Several observations may be related to this proposal. First, pretreatment of cells with insulin results in an increase in calcium bound to subsequently isolated adipocyte plasma membranes¹⁸ and skeletal muscle sarcolemma¹⁹. Increased cytoplasmic calcium resulting from the inhibition of a calcium pump may regulate the availability of calcium for binding to specific sites on the plasma membrane. This increase in bound calcium could be responsible for the known effect of insulin to hyperpolarise muscle²⁰ and fat cells²¹, as calcium has been shown to influence membrane permeability^{22,23} and the membrane potential^{24–26} of other electrically inexcitable cells. Second, the observations reported here could

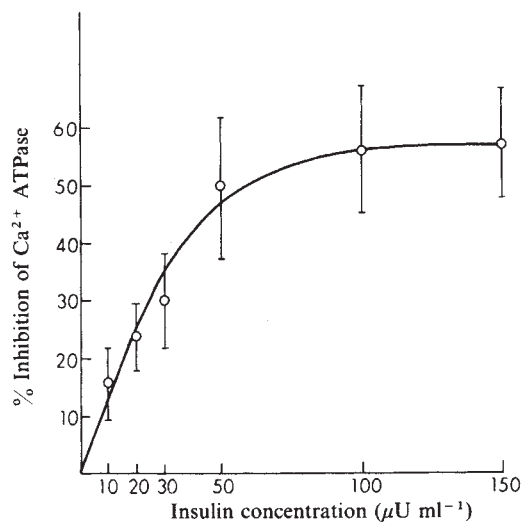


Fig. 2 Concentration-dependent inhibition of the high affinity Ca^{2+} -ATPase by insulin. Assay conditions were identical to those described in Fig. 1 and Table 1. Each point represents the mean $\pm$ s.e.m. of results obtained from four different membrane preparations. Free $[\text{Ca}^{2+}]$ is $3.5 \times 10^{-8} \text{ M}$.

also relate to insulin's regulation of glucose transport and membrane phosphorylation. Both membrane-bound calcium²⁷ and membrane phosphorylation²⁸ have been implicated in the regulation of glucose transport, and ATP has been reported to have a permissive effect on insulin-stimulated glucose transport²⁸. Also, direct addition of insulin to these adipocyte plasma membranes has recently been shown to specifically inhibit the phosphorylation of two protein bands²⁹. One is a 120,000 molecular weight (MW) plasma membrane protein; the other a 42,000-MW protein which originates from the small amount of mitochondria present in the preparation and has been identified as the α subunit of pyruvate dehydrogenase, an insulin-sensitive enzyme²⁹. The unidentified insulin-sensitive 120,000 MW plasma membrane protein could represent the high affinity Ca^{2+} -ATPase as the analogous red cell Ca^{2+} -ATPase phosphoprotein has a similar molecular weight³⁰.

Table 1 Inhibitory effect of insulin on Ca^{2+} -ATPase activity at different free calcium concentrations*

Free $[\text{Ca}^{2+}]$	Ca^{2+} -ATPase activity (nmol ^{32}P mg $^{-1}$ min $^{-1}$)		% Inhibition	Significance
	Control	+Insulin		
4.2×10^{-8} M	28.7 ± 6.7	16.9 ± 4.4	43.1 ± 7.7	$P < 0.01$ ($n = 9$)
7.1×10^{-8} M	49.6 ± 8.0	38.4 ± 7.3	25.1 ± 9.8	$P < 0.02$ ($n = 8$)
1.6×10^{-7} M	104.1 ± 19.1	92.4 ± 17.5	13.3 ± 3.1	$P < 0.01$ ($n = 7$)

* Assay conditions were identical to those in Fig. 1. Insulin was added to a final concentration of $100 \mu\text{U ml}^{-1}$ from a stock solution containing 0.1% bovine serum albumin (BSA). The final BSA concentration in each assay was 0.00125%. Controls contained an identical concentration of BSA. Results are expressed as the mean $\pm$ s.e.m. of n different membrane preparations. Significance was determined using the two-tailed paired T -test.

Increased cytoplasmic calcium resulting from inhibition of a calcium extrusion pump could directly regulate a variety of insulin-sensitive calcium-dependent enzymes including glycogen synthetase³¹, hormone-sensitive lipase³² and pyruvate dehydrogenase³³. Alternatively, this increased calcium could mediate a variety of intracellular processes via binding to a calcium-dependent regulatory protein, which has recently been identified in the cytoplasm of adipocytes³⁴.

Thus these data demonstrate a direct effect of insulin on a high affinity Ca^{2+} -ATPase in the plasma membrane. Although further work is necessary to elucidate its role in insulin action and the mechanism whereby insulin inhibits this enzyme, our observations imply an important and fundamental role of calcium in the mechanism of insulin action.

We thank Dr Barry Siegfried for discussions and Becca Hokanson for technical assistance. Supported by USPHS grants AM20579, AM25897, and ES07066. H. A. P. is a recipient of a Juvenile Diabetes Foundation Research Fellowship.

Received 18 July 1979; accepted 14 August 1979.

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Evidence for a charge-shift electrochromic mechanism in a probe of membrane potential

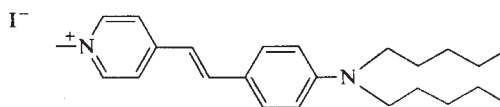
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Extrinsic optical probes have become important tools for monitoring membrane potential, with probes now available for many tissue or cell suspension systems¹. In each case that has been studied in detail^{2–4}, it seems that the mechanism involves a shift in the equilibrium population of the probe from one chemical environment to another in response to the transmembrane potential; the environments perturb the probe's spectrum differently. As this indirect mechanism involves a redistribution of dye between chemical environments that are likely to vary if a given probe is transferred from one membrane to another, a potential probe that is effective and calibrated for all membrane systems has not been realised. We present here evidence for a direct response of a probe chromophore to the electric field across membrane systems. The results suggest it might be possible to develop a universal set of membrane probes.

The probe used is the first to emerge⁵ from a recently developed theoretical methodology⁶. According to this theory, the probe, 4-[p-(dipentylamino)styryl]-1-methyl-pyridinium iodide (di-5-ASP), has a ground state charge distribution which



favours orientation of the pyridinium ring near the aqueous interface, with the probe long axis orientated perpendicular to the membrane surface. The theory also predicts that, upon photoexcitation, the centre of positive charge will undergo a shift from the pyridinium end to the aminophenyl end of the chromophore. A transmembrane electric field should therefore induce a direct electrochromic shift^{7,8} of the absorption spectrum of di-5-ASP⁹.

The spectral responses of di-5-ASP bound to a hemispherical bilayer membrane of oxidised cholesterol⁹ were used to provide experimental evidence for this predicted mechanism. The apparatus for determination of potential dependent fluorescence changes is shown in Fig. 1. Absorbance changes were determined with an apparatus essentially identical to that previously described for experiments with a planar bilayer³.

An important criterion for electrochromism is that the first derivative of the absorption (or excitation) spectrum closely matches the response spectrum¹⁰. Application of this criterion to di-5-ASP is illustrated in Fig. 2; there is a small deviation,

probably because the most responsive probe molecules are those orientated perpendicular to the membrane and these, in turn, have shorter wavelength absorption¹¹. Figure 2 also illustrates how the probe senses the reversed potential when it is applied to the inside of the membrane. Hyperpolarisation of the membrane causes a blueshift of the spectrum when probe is applied to the inside and a redshift for probe bound to the outer surface, consistent with the behaviour predicted for a charge shift electrochromic mechanism⁶. On the other hand, an 'on-off' mechanism³ would predict the opposite behaviour, as membrane bound di-5-ASP is blueshifted with respect to aqueous di-5-ASP¹¹.

The orientation dependence alluded to above is demonstrated in Fig. 3. The probe has a strong tendency to align perpendicular to the membrane surface¹¹, a feature which was an important design consideration. Fig. 3 shows that the response to potential is even more sensitive to orientation, showing a dichroism of $\sim 80:1$ at 480 nm. This can be easily rationalised in terms of electrochromism, as the small population of probes capable of absorbing light polarised parallel to the membrane would also have a relatively lower interaction with the transmembrane electric field.

A true electrochromic response is instantaneous on the time scale of any event which requires movement of nuclei, such as the development of a membrane potential. We have determined that, within the admittedly slow time scale required for charging of the hemispherical bilayer (100 μ s), this condition is met for the absorption response of di-5-ASP.

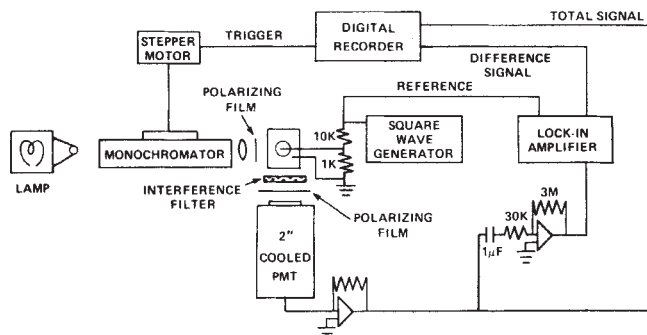


Fig. 1 System for the determination of corrected excitation and potential response spectra from probe-labelled hemispherical lipid bilayers. [System components are: Oriel 100-W quartz-halogen source, powered by Sorensen d.c. supply (DCR40-25B); Instruments SA model H20V monochromator with stepping motor and controller; 570 nm 2×2 inch interference filter (Ditric Optics); EMI-9558QA photomultiplier in Pacific Photometric Instruments model 3463 thermoelectrically cooled housing; Princeton Applied Research 5203 lockin amplifier-tuned amplifier; B&K Precision square wave generator; Bascom Turner 8110 recorder.] A decane solution of oxidised cholesterol⁹ was used to prepare hemispherical bilayers. The solution was lightly brushed along the bottom of a 100 mM KCl solution contained in a 2 mm i.d. polyethylene tube which was attached to a micrometer syringe. The tube was immersed in 100 mM KCl contained in a cuvette, and a hemispherical bilayer was formed via gentle pressure from the syringe⁹. The square-wave potential pulses were applied through Ag-AgCl electrodes in the tube and the cuvette. The appropriate quantity of probe was added to the aqueous KCl as a 3 mM solution in ethanol; the total ethanol concentration was always below 0.3%. (Probe synthesis as in ref. 11). Since membrane-bound di-5-ASP fluoresces ~ 100 times more strongly than aqueous di-5-ASP, fluorescence detection is an effective way of isolating the membrane response; also, the emission interference filter rejects aqueous fluorescence ($\lambda_{\max} = 620$ nm) but passes membrane fluorescence ($\lambda_{\max} = 575$ nm). One of the key components of the system is the digital recorder which allows simultaneous acquisition of response spectra and excitation spectra. A rhodamine B quantum counter was used to generate a calibration curve, stored on the recorder's floppy disk, to correct the experimental spectra for the wavelength dependence of the exciting light intensity.

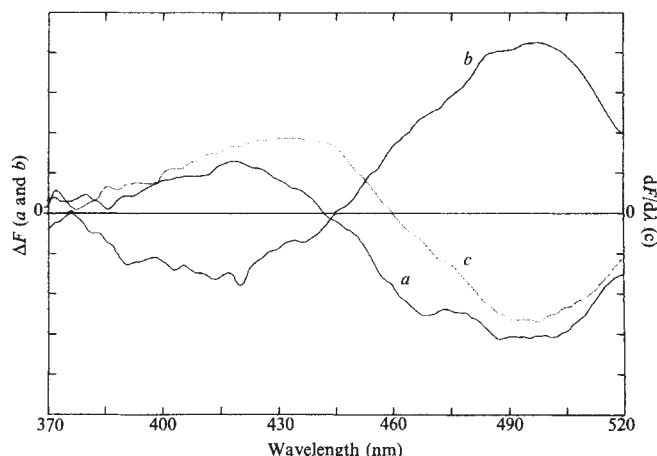


Fig. 2 Corrected response of the probe excitation spectrum to a 100 mV potential pulsed at 200 Hz. Apparatus described in Fig. 1. *a*, 3 μ M probe applied outside the bubble; *b*, 3 μ M probe inside; *c*, first derivative of the corrected excitation spectrum.

In addition to providing positive evidence for charge-shift electrochromism, the experimental results argue against the more common mechanisms²⁻⁴ adopted by potential probes. At low lipid: probe ratios, aggregation of di-5-ASP can be observed in the membrane, detected by a blueshift in the absorbance and a quenching of fluorescence (L. M. L. and L. S., unpublished results); the similarity of the absorption response (Fig. 3) and the excitation response (Fig. 2) spectra, rule out the involvement of these non-fluorescing aggregates in the mechanism. A reorientation mechanism¹² is precluded by the results of Fig. 3; such a mechanism would require responses of opposite sign for the two polarisations. Another possible, and in this case unprecedented, process involves electrophoretic movement of the probe from one membrane site to another, with the two environments sufficiently different to induce a spectral shift. In some recent experiments¹³ we have found essentially identical spectra with an electrically neutral analogue of di-5-ASP; this is strong evidence against the involvement of simple electrophoresis in the mechanism.

Electrochromism will assure a fast, well characterised and, perhaps, uniform response to membrane potential which may provide a special niche for di-5-ASP within the large repertoire of optical probes. Some of its other characteristics will be equally important in determining its usefulness, however. As $\Delta F/F$ is as

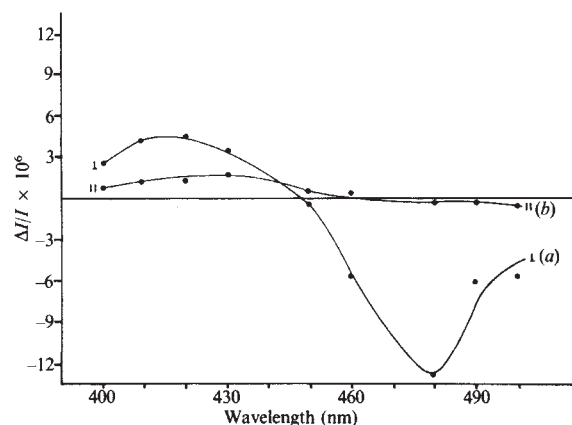


Fig. 3 Response of the absorption spectrum of 5 μ M probe to 100 mV potential steps applied across the hemispherical membrane at 200 Hz. *a*, Incident light focused on the bottom $\frac{1}{3}$ of the bilayer and polarised perpendicular to this approximately planar area; *b*, polarisation parallel to the membrane. The apparatus has been previously described³.

large as 5% for a 100 mV change in potential when exciting near the red wing of the absorption band, it is likely that monitoring fluorescence will provide the best signal. The probe binds strongly to membranes (in lipid vesicle experiments¹¹ we were unable to detect unbound probe even at a 1:1 lipid:probe ratio). The membrane-probe fluorescence is blueshifted by ~40 nm (to 575 nm) and is ~100 times more intense relative to aqueous probe; this provides obvious practical advantages in some applications, but would lower the relative change in fluorescence in an axon experiment, for example, as a result of high background fluorescence from the Schwann cells. The photostability of di-5-ASP appears good, a 3 mM ethanol solution having survived 2 years on an open shelf, so that high intensity excitation could be used to reduce shot noise; the question of photodynamic damage, however, is still open.

Di-5-ASP was the first 'charge-shift' probe to be examined, as much because of its simple preparation as its theoretical promise. The results presented here suggest that it will be worthwhile to develop new charge shift probes which may fit other spectral windows and be even more sensitive. The operation of these probes does not involve a delicate field-dependent balance between different chemical environments, so this approach may provide a universal set of spectroscopic potential probes.

We thank B. Chance, L. B. Cohen, W. N. Ross and J. C. Smith for stimulating discussions. This research was supported by USPHS grants GM25190 and NS10489.

Received 2 July; accepted 10 August 1979.

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The molecular structure of lecithin dihydrate

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Lecithin is a major structural component of biological membranes^{1,2}. Because of their amphipathic nature, lecithin and related phospholipids tend to aggregate as bilayer structures in which the hydrophilic head groups are orientated towards the surface and the hydrophobic hydrocarbon chains towards the interior. A detailed knowledge of the three-dimensional structure of lecithins will aid in the understanding of their role in membrane structure and function, but is still lacking. To this end we have now crystallised and solved the molecular structure of 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC), a lecithin species in the naturally occurring configuration. This compound crystallises from a water-containing solution, with two water molecules (5% w/w) of hydration.

The crystals were grown from commercially available material (Sigma, 98% pure) which had been further purified by recrystallisation from an ether/ethanol/water solution. In this

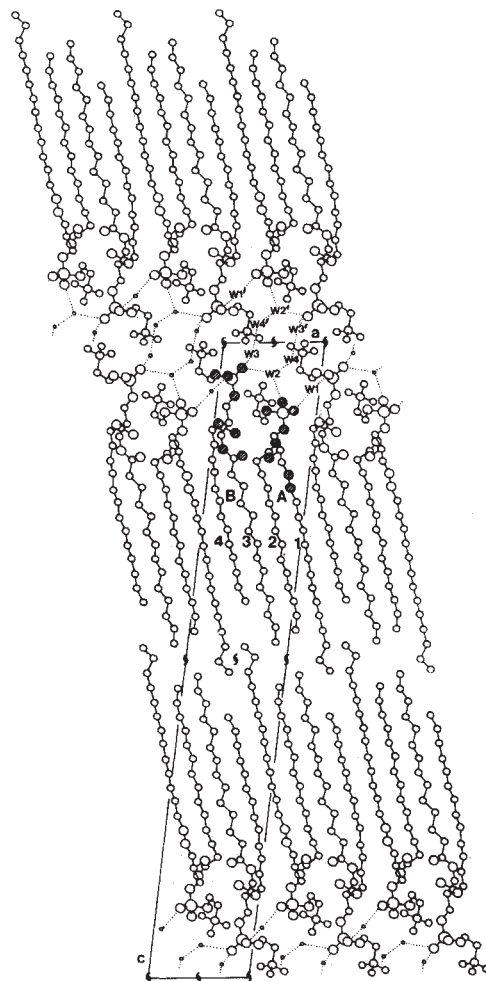


Fig. 1 Molecular arrangement of dimyristoyl lecithin dihydrate projected onto the *ac*-plane. The two molecules A and B of the asymmetric unit are shown with shaded oxygen atoms. The notation of the water molecules of hydration is given for a representative part of the hydrogen bond system (dotted lines) at the polar bilayer interface. The position of the remaining water molecules is indicated by small circles only. The break in the hydrogen bonds at W3' is intended to convey the helical nature of the water structure; connection of this bond leads to the phosphate oxygen of one unit below.

solvent the nucleation of the crystals is accomplished by lowering the temperature of the solution to 14 °C, at which a flocculent precipitate appears. Once nucleated, growth can occur at any temperature between 0 °C and 22 °C, but above 22 °C the crystals redissolve. The crystals are monoclinic, space group P2₁, with two molecules in the asymmetric unit. Unit cell parameters are: *a* = 8.72, *b* = 8.92, *c* = 55.4 Å and β = 97.40°. Reflection data were collected by a peak measurement technique on a GE-XRD6 manual diffractometer. Of the 4,800 reflections thus collected, 1,931 were considered observable by the 2 σ criterion. To maintain the crystals and improve high-angle data quality, the crystals were kept below 20 °C at all times and data were collected at 10–15 °C.

The structure was solved by Patterson and Fourier techniques. The positions of the two phosphorus atoms were found in the Harker sections of the Patterson map and difference Patterson map (where the contribution of the chain carbons had been removed). The remainder of the two molecules in the asymmetric unit was found in successive Fourier maps. Least-squares refinement with isotropic temperature factors (using the Nonius CAD-4 software system for the PDP-11 computer) has reduced the residual factor to 17.4%. The refinement procedure is being continued. An increase in the number of parameters by introducing anisotropic temperature factors is, however, not considered compatible with the number of observed reflections.

The molecules are arranged in a bilayer structure with strong hydrogen bonding in the polar portions of the crystal (Fig. 1). The two molecules (A and B) in the asymmetric part of the unit cell are displaced from one another in the direction of the bilayer normal by 2.5 Å, or one whole zigzag unit of a hydrocarbon chain. Due to this displacement the bulky phosphorylcholine groups require a rather small area (38.9 Å² per molecule) of the layer surface which allows the two hydrocarbon chains to be almost perpendicular (78°) to the bilayer boundary. This type of offset may be related to the displacement of molecules observed in the rippled Pβ' structure of lecithin bilayers in more fully hydrated conditions^{3,4}. Phosphatidylethanolamine (PE), which has a smaller polar group, does not form such rippled bilayers. In the crystal structure of 1,2-dilauroyl-DL-phosphatidylethanolamine (DLPE)^{5,6} the molecules occupy practically the same area of 38.6 Å² in the layer plane but align with their polar groups parallel to the bilayer surface, without a mutual displacement in direction of the bilayer normal. A corresponding arrangement of phosphorylcholine groups which is observed in the crystal structure of the lysolecithin analogue, 1-lauroyl-propandiol-3-phosphorylcholine monohydrate (LPPC)⁷, requires an area per molecule of 52.1 Å². Although there are many common features in the conformation of the molecules in the crystal structure of DLPE and DMPC, a significant difference exists with respect to the interaction between the polar groups. In DLPE the -NH₃⁺ group forms superimposed hydrogen bonds/salt bridges 2.8 Å long with unesterified phosphate oxygens of adjacent molecules. In DMPC (and LPPC) the nitrogen atom of the bulky -N(CH₃)₃⁺ group comes no closer to the phosphate oxygens than 4.5 (3.7) Å. Instead, the non-ester oxygens which share the negative charge are separated and shielded by water molecules of hydration. Two water molecules (W1 and W2) are located between the phosphate oxygens of molecules A and B and thus form an infinite hydrogen-bonded phosphate (A)-water-phosphate (B)-water ribbon (Fig. 1) which provides stability in the *a*-axis direction of the bilayer plane. A similar phosphate-water ribbon is observed in the crystal structure of LPPC. The two other water molecules (W3 and W4) are hydrogen bonded to one phosphate oxygen of molecule B and to water molecule W2, respectively, and establish a linkage across the bilayer interface with the corresponding symmetry-related water molecules (W3' and W4') of the adjacent bilayer. Thus, a helical water structure about the two-fold screw axis is formed (see Fig. 1) which provides structural stability between the bilayers and in the *b*-axis direction of the bilayer plane.

The lateral arrangement of the hydrocarbon matrix is not consistent with earlier known simple chain packing modes. The orientation of the chain planes is alternately parallel and perpendicular to each other and resembles to some extent the hybrid chain packing modes⁸ observed for other double chain lipids. The cross-section of 19.0 Å² per chain also corresponds to that of cerebroside⁹ (19.1 Å²), whereas DLPE is slightly larger (19.3 Å²) due to its shorter chains. Note, however, that the hybrid packing in DMPC is well defined near the carboxyl end only. Towards the methyl end the chain planes are twisted to a

mutually parallel orientation resembling a triclinic parallel (T||) chain packing mode. This successive change in chain orientation indicates the close relationship between this hybrid subcell and the hexagonal chain arrangement with increased rotational disorder, as has been reported for the gel state of fully hydrated bilayers^{3,4}. The small chain tilt of 12° which is observed for DMPC, but not for DLPE (0°), is due to the minute differences in molecular area (+0.3 Å²) and chain packing density (-0.3 Å², see above).

Figure 2 is a detailed picture of the molecular conformation of the two DMPC molecules. The main difference between the two molecules concerns the conformation and orientation of their polar groups. The phosphorylcholine chains are almost mirror images of one another as shown by the torsion angles listed in Table 1. These angles are very similar to those observed in the crystal structures of other phospholipids and phospholipid constituents, which are included in Table 1 for comparison. This preferred conformation common to all phosphorylcholine and ethanolamine compounds reflects the tendency of the ammonium nitrogen to fold back towards the phosphate group so as to minimise the distance between the groups of opposite charge.

The phosphorylcholine groups of the two DMPC molecules make small angles with the bilayer surface. The P-N vectors are 4.3 and 4.5 Å long and inclined to the plane of the bilayer by 17° and 27° for molecules A and B, respectively. The somewhat more extended conformation of the molecule B polar group apparently arises from its more intimate participation in the water structure and its interaction with charged groups of the adjacent bilayer. This stretching and increased inclination of the polar group (which mainly affects torsion angle α₄ (see Fig. 2 and Table 1)) might also be expected in natural membranes when they become associated with ions. The cadmium chloride complex of GPC¹⁰ listed in Table 1 reflects the response of the polar group conformation to the proximity of metal ions, and NMR studies on lecithin in the presence of polyvalent cations¹¹ confirm this.

The most obvious conformational difference between the two DMPC molecules, however, is in the orientation of the polar groups with respect to the hydrophobic parts. In molecule A the phosphorylcholine group points away from its own diglyceride part, but is close to it in molecule B. These two orientations are due to different conformations about the C₍₃₎-C₍₂₎ bond of the glycerol moiety (see Fig. 2 and Table 1). The torsion angle, θ₁, about this bond is +58° (+*gauche*) and +169° (*antiplanar*) in molecules A and B, respectively. In DLPE another staggered conformation (θ₁ = -52°; -*gauche*) is adopted whereas in LPPC an eclipsed *synplanar* conformation (θ₁ = 28°) is approached. This shows that there are no preferred conformations about the C₍₂₎-C₍₃₎ bond. This bond apparently allows rotational independence between the asymmetric (1,2-*sn*-configuration) and conformationally similar diglyceride moiety (see below) and the polar group which exists as mirror image conformers. Results from deuterium NMR studies¹² indicate that in both the gel and liquid-crystalline states of lecithin, such a rotation of the polar group takes place, and seems to be accompanied by a rapid

Table 1 Torsion angles of crystallographically determined phospholipid constituents*

		α ₁	α ₂	α ₃	α ₄	α ₅	θ ₁	θ ₃	β ₁	β ₂	β ₃	β ₄	γ ₁
1,2-dimyristoyl- <i>sn</i> -glycero-3-phosphorylcholine.2H ₂ O(DMPC)	A	162	68	63	139	-51	58	-176	76	180	-87	63	-173
	B	170	-76	-46	-161	64	169	175	124	169	-126	63	103
<i>sn</i> -glycero-3-phosphorylcholine (GPC) ¹⁵	1	-165	-71	-54	-138	73	172	-63					
	2	-172	64	65	140	-75	-63	-69					
<i>sn</i> -glycero-3-phosphorylcholine CdCl ₂ .3H ₂ O ⁹		168	-69	-73	178	73	171	59					
1-lauroyl-propandiol-3-phosphorylcholine.H ₂ O (LPPC) ⁷		162	86	45	129	84	28	78					156
1,2-dilauroyl- <i>rac</i> -glycero-3-phosphorylethanolamine.CH ₃ COOH (DLPE) ^{5,6}		-154	58	66	106	67	-52	-172	97	179	-119	65	-178
<i>sn</i> -glycero-3-phosphorylethanolamine.H ₂ O (GPE) ¹⁶		-173	-81	-81	163	55	166	-67					

Remaining β and γ torsion angles are approximately *antiplanar* (180°).

* For notation of torsion angles see Fig. 2 legend.

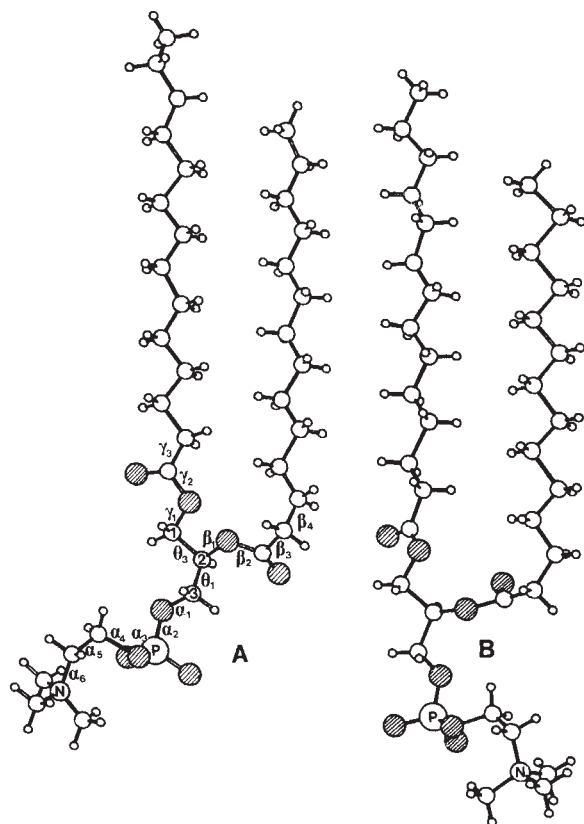


Fig. 2 Molecules A and B of dimyristoyl lecithin in a view along the unit cell *a*-axis. Hydrogen atoms are added at theoretically calculated positions to improve the perspicuity. The notation of torsion angles according to Sundaralingam¹⁷ is indicated for molecule A. The figure illustrates the mutual 2.5-Å displacement of the two molecules in the direction of the hydrocarbon chains and the 3.7-Å offset between the two hydrocarbon chains within each molecule caused by the characteristic chain stacking.

inversion between enantiomeric conformations. The DMPC molecules A and B and also DLPE and LPPC obviously represent different energetically equally favoured stages of the freely rotating polar group.

In spite of the different orientations and mirror image conformations of the polar group, the diglyceride part is very similar in both DMPC molecules (Fig. 2). The three glycerol carbon atoms and the *sn*-1-fatty acid together form an *antiplanar* zigzag chain which is only slightly inclined (12°) from the layer normal. The initial part of *sn*-2-fatty acid extends perpendicularly (layer parallel) from this straight backbone but bends off at the second carbon atom to become parallel to the *sn*-1-chain. The same type of chain stacking is found in the DLPE crystal and, according to recent NMR studies¹³, is also adopted by lecithin and phosphatidylethanolamine in hydrated bilayers and *Acholeplasma laidlawii* membranes. This diglyceride conformation must therefore be considered as a general, preferred feature in membrane phospholipid arrangements. The axial displacement of the two fatty acid chains of 3.7 Å by three methylene groups, which is caused by this chain stacking, is to some extent compensated in natural phospholipids by different chain lengths of the two fatty acids. In egg lecithin¹⁴, for example, the predominant chain lengths in the *sn*-1 and *sn*-2 positions are 16 and 18 carbon atoms, respectively. This will reduce the interdigitation of the hydrocarbon chain ends at the bilayer centre.

In the DMPL structure the carbonyl oxygens of the fatty acid chains do not seem to take part in polar interactions, although the oxygens of the *sn*-2 chains (2 and 4 in Fig. 1) are exposed to the polar group region. These oxygens should become more easily available for interaction if the cross-section of the molecule is increased, due to either increased hydration of the polar

groups and/or expansion of the chain matrix on transition to the liquid-crystalline state, which is the usual condition in natural bilayers. This accessibility of the carboxyl group of the *sn*-2-chain at the hydrophobic/hydrophilic interface of the lipid bilayer may also explain why enzymes which split fatty acids from phospholipids, such as lecithin cholesterol acyl transferase and phospholipases of type A, have mostly evolved a specificity for this *sn*-2-fatty acid.

From available data it is obvious that the characteristic conformational features exhibited by DMPC in the crystal structure are also maintained in less ordered lecithin structures in an aqueous environment. The detailed three-dimensional structure presented here provides the necessary basis for a more conclusive interpretation of biochemical phenomena and physicochemical data recorded on model systems and natural membranes.

We thank Professors S. Abrahamsson and A. Hybl, and Drs W. Duax and S. W. Hui for assistance. DMPC was obtained from Dr D. Dorset, and the diffractometer made available by Dr Parthasarathy. Funds for computer usage were in part made available through NIH grant GM-19684 to the Medical Foundation of Buffalo.

Received 2 February; accepted 8 August 1979.

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Heat-shock induced proteins present in the cell nucleus of *Chironomus tentans* salivary gland

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The heat shock (HS) system has been largely studied in *Drosophila* but the molecular mechanisms responsible for the induction of the heat shock genes as well as the function(s) and the intracellular localisation of the induced proteins is still unknown. It has previously been shown that the HS puff induction is accompanied by a local increase of nuclear non-histone proteins (NHP) but the nature of most of the proteins accumulating is unknown¹. We have investigated the effects of a heat shock on *Chironomus tentans* salivary glands, a system where it is possible to study constituents in various subcellular or intranuclear regions including individual puffs^{2,3}, by microdissection. We report here evidence that at least two of the polypeptides synthesised in response to the heat shock migrate to the nucleus. Furthermore, these two proteins appear to have a broad intranuclear distribution, as shown by their presence in the various microdissected nuclear fractions.

At the cytoplasmic level, the heat shock (39 °C) response of *Chironomus tentans* salivary glands is similar to that observed in *Drosophila* (Fig. 1a, b). Several new proteins appear (arrows,

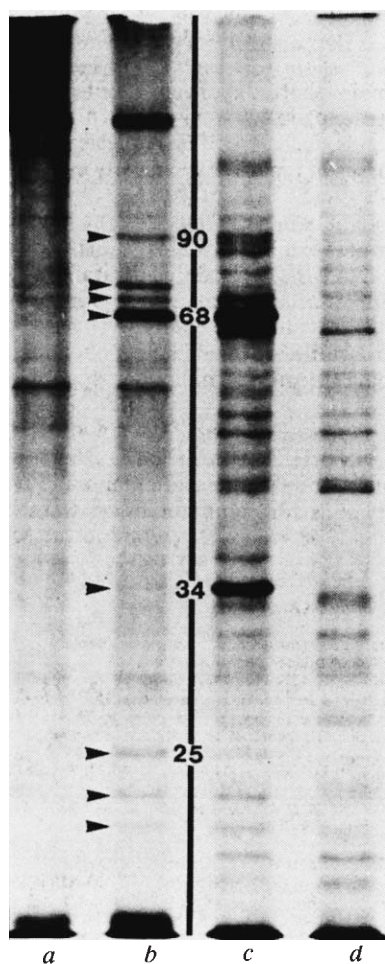


Fig. 1 Photograph of a fluorogram showing the labelled proteins from the cytoplasm of control *a* and heat-shocked larvae *b* and from the nuclei of heat-shocked *c* and control larvae *d*. Larvae were incubated at 39 °C for 10 min, and control larvae at the same developmental stage, were kept at room temperature. For both treatments, 10 salivary glands were excised and incubated for 90 min in 50 μ l Cannon's modified medium (without leucine) supplemented with 100 μ Ci 3 H-leucine. After fixation of the glands in absolute ethanol:acetic acid (3:1), the glands were microdissected to obtain pure intracellular compartments. The proteins from the pooled components were extracted and analysed on a 8.75% acrylamide-SDS slab gel as described elsewhere^{4,5}. The labelled polypeptide bands were revealed by fluorography⁷. In this experiment, 200,000 c.p.m. of each microdissected extract were put on the gel which had been exposed for 3 d. Arrows on *b* indicate cytoplasmic HS peptides. Approximate molecular weights, in kilodaltons are indicated in the middle of the photograph.

Fig. 1b) with a major one of molecular weight (MW) about 68,000. (R.M.T. and M.V., in preparation.) This protein apparently has the same MW as the major heat-shock induced peptide in *Drosophila* cell cultures and salivary gland cells. Also, normal protein synthesis is repressed in response to a 39 °C heat shock (by ~50%), as in *Drosophila*. Thus, even though the sites and numbers of heat-shock induced puffs have not been studied in *Chironomus*, it seems that its response at the protein synthesis level is quite similar to that of *Drosophila*. *In vivo* treatments (5–20 min at 39 °C) gave similar results with the exception of a gradual decline in total protein synthesis. More drastic treatments, such as a 40-min shock at 39 °C or a 10-min shock at 41 °C, resulted in an almost complete shutdown of protein synthesis (data not shown).

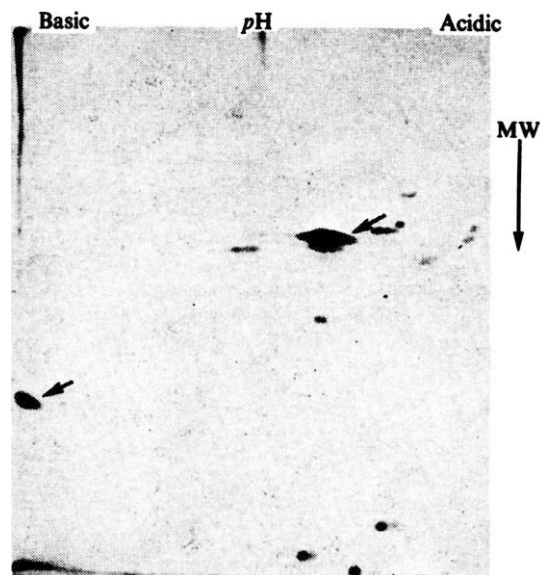


Fig. 2 Photograph of a two-dimensional fluorogram showing the labelled proteins from the nuclei of heat-shocked larvae. Iso-electric focusing in the first dimension was carried out in 9.5 M urea and SDS-electrophoresis in the second dimension was on a 8.75% acrylamide gel as described by O'Farrell⁸.

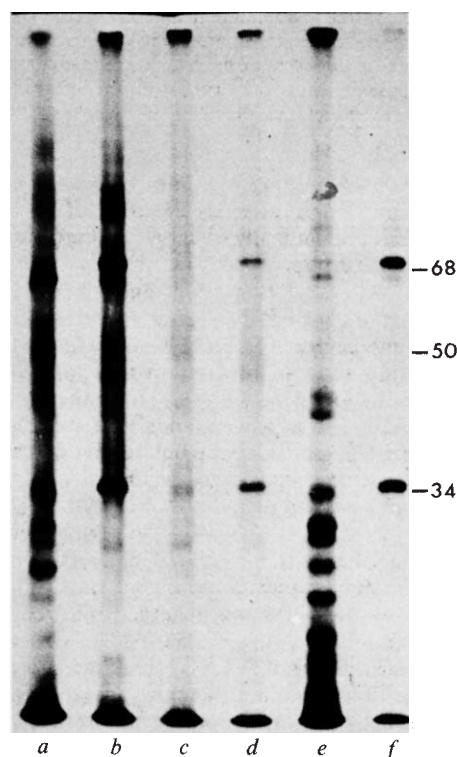


Fig. 3 Fluorogram of newly-synthesised proteins from sub-nuclear microdissected compartments. *a* Chromosomes from control glands; *b* chromosomes from heat shocked glands; *c* nuclear sap, control; *d* nuclear sap, heat shock; *e* nucleoli, control; *f* nucleoli, heat shock. Molecular weight markers were bovine serum albumin (68,000), γ -globulin heavy chain (50,000) and tropomyosin (34,000). Experimental procedure as described in Fig. 1 legend.

In a search for possible nuclear modifications accompanying the heat treatment, we analysed newly synthesised proteins from microdissected nuclei (Fig. 1c, d). The nuclear protein pattern from treated cells shows two major peaks, at MW 34,000 and 68,000 (Fig. 1c), not found in the control nuclei, the latter migrating similarly to the major cytoplasmic protein. However, the 34,000-MW protein, while being a major component of the HS nucleus, is barely visible on the HS cytoplasmic profile (Fig. 1b), suggesting a rapid migration of the polypeptide to the nucleus.

On the other hand, the two-dimensional pattern of the heat-induced nuclear proteins (Fig. 2) clearly established that these two peptides have distinct isoelectric points, arguing against a precursor-product relationship between them. These results provide the first indication for a nuclear role of some of the HS induced proteins.

Several minor bands on the HS cytoplasmic and nuclear profiles (Fig. 1b, c) are common to both of them. These could represent additional HS proteins which are also transferred to the nucleus. However, they could also result from minor contamination of the dissected nuclei with cytoplasmic material.

We examined the intranuclear localisation of these two proteins using microdissection techniques. The nuclei were dissected into three fractions—chromosomes, nucleoli and nuclear sap—and the proteins from each compartment were extracted and analysed by SDS-polyacrylamide gel electrophoresis as previously described^{4,5}. In addition to containing the chromosomal proteins present in the control (Fig. 3a), the chromosome fraction from heat-shocked cells (Fig. 3b) shows

two extra proteins with MWs of 68,000 and 34,000. These two proteins may be present in the HS-treated nuclear sap (Fig. 3d) simply because they are passing through it, but the possibility that they have some function there cannot be excluded. Finally, the nucleolar pattern shows some interesting features: the low molecular weight proteins characteristic of control nucleoli (Fig. 3e) are absent in the treated gland nucleoli, while the two HS-induced polypeptides are heavily represented in this fraction (Fig. 3f). In summary, these results show the migration to the nucleus of at least two newly synthesised HS-induced proteins which appear to have a general intranuclear distribution.

This provides new information about these proteins and suggests a nuclear role for some of them. There has also been an indication that HS proteins are present in *D. melanogaster* nuclei⁶. These proteins may be involved in a general mechanism responsible for the functional or morphological maintenance of the HS nucleus or in various processes such as the silencing of preexisting puffs and the transport or processing of the induced mRNAs.

We thank Mrs F. Biron for technical help and the MRC of Canada, grant MA-5278 to R.T. and a studentship to M.V.

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Quaternary structure-induced photoreduction of haem of haemoglobin

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Spectroscopic studies have provided extensive information on the primary process of visual pigments and photoexcitation of chlorophyll as well as their effects on the surrounding polypeptide chain, but a dependence of photoreactivity on the higher-order structures of protein has been observed only rarely. Resonance Raman spectroscopy can reveal the vibrational frequencies of the chromophore in a molecule provided the excitation wavelength is in the absorption band of that molecule. As the visible absorption bands of haemproteins are due to $\pi\pi^*$ transitions of the porphyrin ring, we can selectively observe the vibrational frequencies of iron porphyrin during *in situ* interactions with immediate amino acid residues of protein when the wavelength of excitation light is close to the Soret or Q band. Correlation of some vibrational frequencies of haem with the oxidation and spin states of the haem iron has been studied in detail and an empirical rule has been established^{1,2}. This method is therefore especially suitable for the study of an effect of higher-order structures of protein on the chromophore. We report here a photoreaction facilitated by a particular quaternary structure of protein—in various haemoglobins resonance Raman spectroscopy showed that reversible photoreduction of haem took place in the T state but not the R state.

Haemproteins containing iron porphyrin as a prosthetic group function as oxidases, oxygenases, carriers and reservoirs of oxygen, or electron carriers in a respiratory chain in most organisms. Haemproteins do not normally serve as photorecep-

tors—photodissociation of axial ligands such as O₂, CO and NO bound to the Fe²⁺ ion was previously the only known photoreaction of haemproteins³. Methaemoglobin has been thought to be photostable, but during resonance Raman studies of the quaternary structures of haemoglobin (Hb), we found that a haem of some met-Hb in the T quaternary structure (low affinity for oxygen), but not in the R quaternary structure (high affinity for oxygen)⁴, was extensively photoreduced on irradiation by laser light at 441.6 nm. The apparent photoreduction of haem was reversible and dependent on the wavelength of the irradiated light.

Figure 1 shows the resonance Raman spectra of various Hb molecules in the frequency region 1,200–1,700 cm⁻¹. It is well established that in this frequency region the resonance Raman spectra of the two oxidation states are distinctly different^{1,2}. Deoxy-Hb (Fig. 1a) and aquomet-Hb (Fig. 1c) exhibited the characteristic Raman lines of their oxidation states at 1,355 and 1,371 cm⁻¹, respectively. When inositol hexaphosphate (IHP), an effector stabilising the T structure, was added to aquomet-Hb, prominent Raman lines appeared at 1,371 and 1,355 cm⁻¹ (Fig. 1b). Furthermore, extra Raman lines were also observed in a lower frequency region (not shown) and their frequencies (366, 341, 302 and 216 cm⁻¹) coincided with those of deoxy-Hb⁵. In the frequency region above 1,500 cm⁻¹, relative intensities of Raman lines were altered by laser irradiation and the intensified Raman lines reasonably corresponded to the Raman lines of deoxy-Hb. These facts suggested that the haem of aquomet-Hb in the T structure was partially photoreduced by laser irradiation.

Carp aquomet-Hb in the presence of 2 mM IHP is known to adopt the T structure⁶: in a stationary cell this gives the spectrum shown in Fig. 2d. There were Raman lines at 1,355 and 1,371 cm⁻¹, as for human aquomet-Hb, implying that carp aquomet-Hb in the T structure was also partially photoreduced. When the same sample was measured in the spinning cell (1,800 r.p.m.), the spectrum (Fig. 1e) was indistinguishable from that observed in the absence of IHP. It was previously noted that photodissociable haemproteins were apparently protected from photodissociation in the spinning cell⁷. The actual mechanism involves recombination of the ligand during passage of the

molecules through the dark. Thus it is probable that in the spinning cell the photoreduced Hb re-oxidised while it passed through the dark. As in the spinning condition a Hb molecule was irradiated by laser light for 0.3 ms every 30 ms, the time required for re-oxidation must be less than 30 ms. The alternative would be that the photoreduction did not take place within 0.3 ms.

Figure 2 shows the Raman spectrum of carp aquomet-Hb in the presence of IHP measured at three different power levels of laser light. The Raman line of the photoreduced species at $1,355\text{ cm}^{-1}$ became relatively more intense when the power level was increased. A similar phenomenon was observed by Cheung *et al.*⁸ for etioporphyrinato-Ni(II) excited at 404.6 nm. They reported that tight focusing of the incident laser light at the sample point resulted in a frequency shift of a Raman line from $1,384$ to $1,375\text{ cm}^{-1}$ and weakening of other Raman lines. The present observation differs in that two Raman lines were observed simultaneously—one at $1,371$ and one at $1,355\text{ cm}^{-1}$. The line at $1,371\text{ cm}^{-1}$ did not completely disappear even in an anaerobic condition and the intensity ratio, $1,371/1,355\text{ cm}^{-1}$, was unaltered by prolonged irradiation (up to 1 h). The photoreduced species was not recognised on irradiation of laser light at 488.0 and 514.5 nm. Other met-Hb derivatives including fluomet-Hb, azidomet-Hb and cyanomet-Hb were almost equally photoreduced when IHP was present at neutral pH. All these facts indicated that photoreduction of the haem of met-Hb apparently took place when met-Hbs were in the T quaternary form but not in the R form, and that the photoreduction was reversible. Furthermore, the lifetime of the photoreduced species was extremely short.

Photoreduction of haem, similar to that reported here, was recently observed for cytochrome oxidase on irradiation of laser light near the Soret band⁹⁻¹¹; the electron donor was thought to be a flavin impurity. In the present case, however, the Hb solutions used for the T and R forms were identical except for the presence or absence of IHP. Therefore the electron donor for photoreduction is endogenous and may possibly be axial

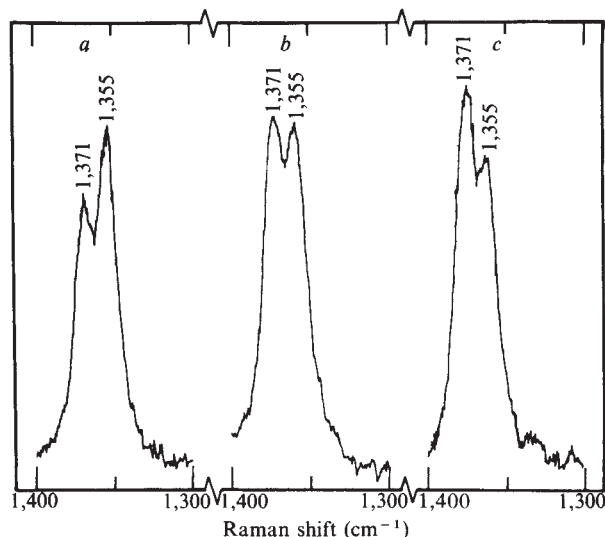


Fig. 2 Raman spectra of carp aquomet-Hb in the presence of 2 mM IHP in the frequency region $1,300\text{--}1,400\text{ cm}^{-1}$. The Raman spectra were excited by the 441.6 nm line of He-Cd laser at the power level of a, 12 mW; b, 3 mW and c, 1.5 mW. Laser light was attenuated by the 1/4 and 1/8 ND-filters.

ligands of haem iron. Whatever the electron donor involved, this study suggested that an electron was reversibly transferred to haem from the donor only in the T structure while the oxidation state of the whole molecule remained unaltered, and that the rate of electron transfer depended on the wavelength of irradiated light, presumably as a result of the wavelength-dependent electronic excitation of the acceptor group.

We thank Kinmon Electric Co. for the use of a He-Cd laser, and Mr T. Ogawa for providing carp.

Received 29 May; accepted 10 August 1979.

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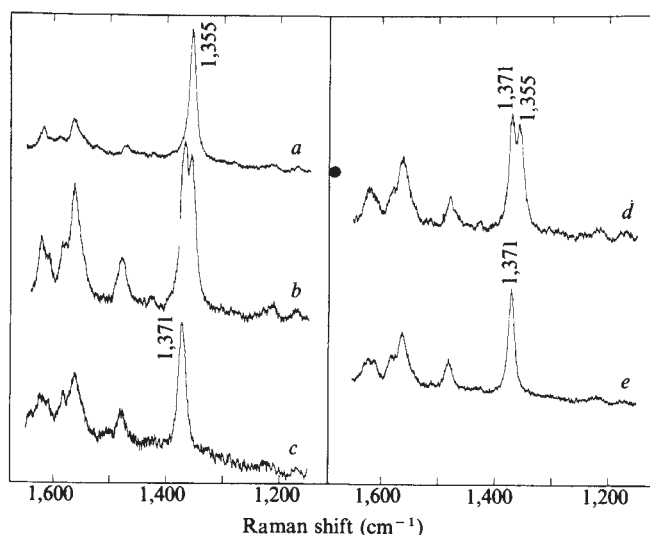


Fig. 1 Resonance Raman spectra of human and carp aquomet-Hbs in 0.05 M bis-Tris, 0.05 M Tris buffer pH 6.5 excited at 441.6 nm (He-Cd laser, Kinmon Electronics). a, Human deoxy-Hb A; b, human aquomet-Hb A in the presence of 2 mM IHP; c, human aquomet-Hb A in the absence of IHP; d, carp aquomet-Hb in the presence of 2 mM IHP measured in a stationary cell; e, the same sample as (d) but in a spinning cell (1,800 r.p.m.). Human and carp haemoglobin were prepared as described by Kilmartin and Rossi-Bernardi¹² and Tan *et al.*¹³, respectively. Hb was oxidised at pH 6.9 by addition of 1.2 equivalence of $\text{K}_3\text{Fe}(\text{CN})_6$ and then was stripped of phosphate by passage through a Dintzis column.

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reviews

Members of the club

Thomas H. Jukes

The Eighth Day of Creation: The Makers of the Revolution in Biology. By H. F. Judson. Pp. 686. (Simon and Schuster: New York; Cape: London, UK, 1979.) \$15.95; £7.95.

THE book is in three sections. The first deals, in much detail, with events leading to the discovery of the structure of the DNA molecule. It is a comparatively straightforward topic. The third section is about the structure and function of protein molecules, and the procedures, especially crystallography, that were used in research with proteins. This subject, quite complex for the average reader, lends itself to logical treatment, and the author attacks it with obvious relish. He was helped by his friendly relationship with Max Perutz.

The intermediate section is about molecular biology from the time of the discovery of the DNA formula to the solution of the genetic code. This is a difficult and controversial era for evaluation because of the involvement of many scientific groups, and this is therefore the most personalised and subjective part of the book.

The challenge to genetics and evolution, posed by Watson and Crick in 1953, was a biochemical one: to find out how information travelled from the gene to the phenotype. As described by Judson, enter George Gamow, an ebullient and articulate cosmologist. There followed a strange period in which the mysteries of molecular biology were planned to be solved by a special coterie called the "Tie Club", the membership of which was limited to 24, one for each nucleotide in DNA and each amino acid. Crick was the scoutmaster of the troop, and merit badges (ties and tie clasps) were awarded and issued by Gamow. Apparently, this cabal took itself seriously, and exchanged "Tie Club" letters, on scientific concepts and developments.

All who have followed molecular biology have impressions and memories of the years (1953–1965) between the announcement of the DNA helix and the completion of the genetic code. I suppose that leadership in science is based on a blend of performance and eloquence. Certainly the scientific stars who dominate Judson's narrative were great talkers, with dominant personalities. Francis Crick can hold a packed auditorium spell-bound by a loop of string and a description of supercoiling and writhing. No wonder Judson



George Gamow, about 1954

was swept away, and he tends to be reverent in the presence of the great. For example (page 92):

"We now have standardized people with respect to their intake by giving them a standardized small-molecule diet" — he gestured towards the packet of Vivonex 100 — "which is immediately absorbed from the stomach into the blood stream, no digestion, the contents of the gut quickly disappear and the bacteria too. They are starved to death on this diet. Perfectly nutritious . . ."

This prestidigitation by Pauling was swallowed by Judson, together with the Vivonex, and 2g of vitamin C. He says: "I'd been captured by that infectious grin and that unquenchable self-confidence . . .". Self-confidence does not change the stomach wall, which, in the absorption of food, "behaves as if it were made of porcelain". If not, the liver would be promptly marinated by hydrochloric acid secreted in the gastric juice. Bacteria do *not* disappear from the gut when food is withheld.

A dinner at King's College (1971) inspires Judson to unctuous prose (p221):

From my right, along the polished wood, there arrived a little trolley train on wheels, in the shape of a silver swan, with three parts joined by swivels, and each part bearing a decanter. A card said that the port was Taylor 1955, the white was Raventhaler Herberg Riesling Spatlese Cabinet 1959, and the red was Corton Les Maréchaudes 1962. Beyond Brenner sat a gray-haired woman, a computer mathematician from one of the women's colleges.

Brenner leaned towards her. "There will be no difficulty in computers' being adapted to biology", he said, with clenched teeth. "There will be Luddites. But they will be buried".

Perhaps Sydney was getting a bit edgy about public objections to genetic manipulation.

Dounce's paper on coding (*Enzymologia*, 15, 251; 1952) is given courteous but somewhat brief treatment on pages 247–248. His proposal was that groups of three consecutive bases in RNA each furnished the code for an amino acid; and Yčas (in *The Biological Code*; North-Holland: Amsterdam and London, 1969) states that Dounce " . . . also suggested that the information in the RNA template may be stored in DNA. 'If . . . genes are composed of deoxyribonucleic acid, then the deoxyribonucleic acid gene molecules would act as templates for ribonucleic acid synthesis, and . . . the ribonucleic acid synthesized on the gene templates would then in turn become templates for protein synthesis in the nucleus or cytoplasm or both'. Dounce's proposal thus clearly stated what became known later as the 'sequence hypothesis', as well as the messenger RNA concept."

Even though Dounce's proposal preceded 1953, Judson devotes excessive space to free-swinging and palpably erroneous speculations on coding by Gamow. It must have been a blow that Nirenberg and Matthaei were not

"members of the club" (p447), for according to Meselson, results by non-members were "unlikely to be correct" (p481). However, recovery from the shock was rapid.

Of course, it was the announcement by Nirenberg and Matthaei, and its consequences, that finally brought genetics within the grasp of biochemistry. Judson's criticisms (p487) that the experiments with random polynucleotides failed "to allow for synonyms" are not well taken. The degeneracy of the code was shown by the "shared doublets" and most of the coding assignments were correct; except that the sequence of bases in each triplet was not discernible until later. An epilogue soon followed, although Judson does not mention it: Fred Sanger got tired of waiting for his turn to use the amino acid analyser, so he decided to sequence nucleic acid molecules. (He had previously shown everyone how to sequence proteins.) Place the amino acid sequence of a virus coat protein beside the sequence of the viral RNA molecule, and there you have the complete story — the direction of reading and all the codons. But, apparently, Fred spends too much time in the lab, and not enough in talking to historians.

Judson gives the genetic code the most perfunctory treatment of any major topic discussed, except for long summaries of early theoretical work that turned out to be in vain. The first preliminary indication of code composition was by Sueoka (1961) who showed that high A + T in DNA of bacteria was correlated with isoleucine, lysine, phenylalanine and tyrosine; high G + C with alanine, arginine and glycine in bacterial proteins.

Crick showed great insight in insisting that exactly 20 amino acids were used in protein synthesis (pp252-258). For years afterwards, biochemical authors still frequently talked of "about 20", or "about 22". Hydroxyproline in gelatin was particularly confusing. Asparagine and glutamine were often omitted although isotopic experiments had shown they were incorporated intact. Judson errs in stating



that Gowland Hopkins, in 1901 "had been first, with tryptophan, to isolate an amino acid". Glycine was isolated from gelatin hydrolysate in 1820; tryptophan was seventeenth.

The story of the messenger concept is developed in detail. I well remember the excitement caused by the "DNA-like RNA" finding by Volkin and Astrachan in 1956. The statement that this work "could not quite be dismissed as the inconsequential and self-admittedly sloppy work of people not well known at a lab not highly regarded" (p325), is an unjustified slur on the lab where Cohn and Volkin (*Nature*, 167, 483; 1951) pioneered on phosphate linkages in nucleic acids. Also in 1956, Fraenkel-Conrat, and Schramm, showed that tobacco mosaic virus RNA was by itself infective and carried all the information needed to make the virus. In 1960 (pp432-446) various efforts succeeded in establishing the existence of messenger RNA. However, in 1961, messenger RNA was upstaged by polyuridylic acid, and we learn (pp475-479) that Nirenberg and Matthaei had never even heard of messenger RNA!

Judson, to some extent, follows Watson's style in describing personal characteristics of superstars. The less ostentatious scientists come off second best. Judson says (p581): "what Pardee lacked, perhaps, was the knack of generalization". For "generalization", read "self-glorification". Stent, who is a sort of Louella Parsons of molecular biology, is quoted (p405) as saying that Pardee is its "Douanier Rousseau", an analogy that I found strange. Pardee is probably more of an originator (like Chagall?) than a primitive. Judson's preoccupation with the personal life of his luminaries sometimes becomes excessively chatty. For example (p283):

"On 6 December 1954, Stent wrote to Brenner, saying, in part:

Dearest Sydneyboy!

The Lwoffs have just left, and I have a few moments between cocktail-parties to dash off a couple of lines, so that there'll be news from us awaiting you when you make your triumphant return to the Rand. Ta ever so for your Oxonian and Cantabrian letters which both Inga and your serviteur devoured with the greatest of that old gusto. Boy do we miss you here! You simply *must* come back to Berkeley. In future letters, I hope we can

discuss in more detail just what we can do to bring you and your gang for an extended stay back to Bagdad-by-the-Bay . . .

So, Wolfgang Amadeus von und zu Witwatersrand, receive thousands of amitiés from your ever-faithful

Inga and Gunther"

But Judson does well with prolonged and detailed scientific descriptions in a variety of fields, especially crystallography and protein structure. His book will be widely read by scientists; and of course, the handful who are apotheosised by it will love it, as will their admirers.

Throughout the book, one becomes aware of a race between two schools — on one hand, deductions made by molecular geneticists and theorists, and on the other, the staunch empiricism of biochemists. The fine structure of the gene was solved by Ingram's demonstration in 1956 that glutamic acid at the sixth residue of β haemoglobin was replaceable by mutation to either valine or lysine. Benzer's approach to this was by genetic analysis of rII mutants in bacteriophage T4, which reached some kind of a peak in a six-page fold-out that was inserted in *Proc. natn Acad.Sci. U.S.A.* in 1961. The inexorable progress of biochemistry brought the tangible emergence of transfer RNA, soon following its prediction by Crick's brilliant adaptor hypothesis. On p341, an account of the genesis of the RNA cloverleaf structure would have been welcome. The formula for glucose on p378 is incorrect.

In this book, some of the prominent scientists whose personalities were familiarised by Jim Watson, continue to cavort as they did in *The Double Helix*, and are joined by other performers. We are spared few details, even unto the length of the hairs in Brenner's eyebrows (up to three inches). But Judson's careful and sympathetic account of the life of Monod, developed at great length, is good reading.

Will *The Eighth Day of Creation* be studied as history, read as a novel, or used as a textbook? My own feeling is that the history of molecular biology deserves, for documentary purposes, a more dispassionate and even-handed treatment by someone who will undertake a task comparable in detail to that of Judson.

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Arthur Pardee, 1960

Switching of globin genes

Cellular and Molecular Regulation of Hemoglobin Switching. Edited by G. Stamatoyannopoulos and A.W. Nienhuis. Pp. 792. (Grune and Stratton: New York, San Francisco and London, 1979.) \$68.50.

THIS multi-authored book contains the proceedings of a conference held in June 1978 which aimed to review the areas of haemoglobin synthesis and red blood cell differentiation pertinent to developmental and physiological 'switching' of globin genes. The sheer volume of information in this book is staggering, and it is difficult in this review to do justice to all 48 articles. The style of presentation was left to individual authors and I found most papers very readable, with many contributors presenting their work in a more general, and generous, way than is usually found in the literature. One disappointing feature of the book is that it lacks a general overview of the type previously published for the meeting (see *Cell*, 15, 307-315; 1978), and this leaves the reader to judge how far the field had progressed in 1978.

The book is divided into three main sections covering the changes in haemoglobin synthesis during development, haemoglobin switching and erythroid differentiation, and the molecular biology of globin genes. The first article by Weatherall clearly describes what is known about the genetics of human disorders that are characterised by the persistent synthesis of fetal haemoglobins after birth, and subsequent papers examine human fetal globin chain synthesis *in vivo*. Using animal models for studying the 'switching' of haemoglobins, the concept of stress erythropoiesis in baboons (De Simone *et al.*) and in sheep (Wood *et al.*) is introduced, leading to a series of articles describing the controls of haemoglobin synthesis in these model systems.

Attention is next focused on erythroid precursor cells with articles showing the hormone influence on differentiation and the differences between stem cells isolated from bone marrow and peripheral blood. Highlights of this section include a review by Eaves on erythropoietic differentiation. Rapid progress has been made in the culture of haemoglobin-producing cells *in vitro* and Nienhuis shows examples of this in an article on 'switching' in cultured sheep stem cells. Ingram proposes a provocative model to explain the possible effect of various chemical inducers on the metabolic events in Friend cells which leads to differentiation and the subsequent accumulation of globin mRNA species.

The final section of the book deals exclusively with the molecular layout of globin genes and includes an elegant description of mouse β -globin gene

arrangement by Leder and an explicit guide to cloning genomic DNA in λ bacteriophage by Lacey *et al.* A series of three papers show how the human globin gene map looked in 1978. Globin mRNA is well reviewed in a series of articles covering such topics as sequence, modification, processing, and developmental changes. In the final part entitled "Control of globin gene expression", Weintraub proposes how classes of nuclear protein may interact with DNA in active genes conferring the conformation required for specific expression; this is the only paper to mention nucleosomes. Young *et al.* show the use of DNase I for probing active globin genes in fetal and anaemic sheep, their results

indicating quite different mechanisms for gene activation and deactivation. Finally Deisseroth and Hendrick explain their results with cell fusion and Anderson *et al.* report the progress in trying to use cell microinjection as a potential assay for those elusive regulatory factors.

I feel sure researchers in the haemoglobin field will find this a valuable reference book. It also offers much to the clinician attempting to understand the newer areas of biology.

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Rotating stars

Theory of Rotating Stars. By J.-L. Tassoul. Pp. 506. (Princeton University Press: Princeton, New Jersey, and Guildford, UK, 1979.) Hardback £25; paperback £9.40.

ROTATION pervades the domains of astronomy at every level: planetary, stellar, galactic, and beyond. This book is devoted to the stellar level. The study, both observational and theoretical, of rotating stars is a growing speciality among astrophysicists. No other reference work in the subject even attempts the breadth of coverage presented in this book. It will be heavily used, not only by specialists, but also by non-specialists looking for a concise and authoritative picture of the status of one or another branch of the subject. The explanations are sufficiently clear and self-contained that it could serve as a graduate-level text.

The first two chapters summarise the historical development and observational background, emphasising the problems confronting theory, thereby motivating the choices of theoretical questions found in the remainder of the book. These are followed by a chapter on hydrodynamics, specialised to the conditions prevailing in stars. This is an especially important chapter. It underlies the rest of the book in the sense that virtually all questions subsequently addressed are formulated as hydrodynamical problems. It is not an easy matter to develop hydrodynamics in one chapter (in fact, certain special aspects of the subject, stability theory in particular, are developed in chapter six). Tassoul does a very good job in my opinion, both in explaining the subject matter and in guiding the reader to the literature. For example, specialists in stellar hydrodynamics have had an unfortunate tendency to ignore the large and related body of material in geophysical hydrodynamics. Tassoul is careful to make

the appropriate references to the geophysical literature.

Chapters four to seven are very much preparatory, theoretical, and hydrodynamical in nature, covering steady-state models of rotating axisymmetric stars, techniques for constructing them, techniques for studying their stability, and some results of a general character regarding their stability. Chapter four, on permanent rotations, collects a number of classical results on the nature of the allowable rotating configurations, many of which appear only in older, relatively inaccessible texts or articles. Some are given in detail, like Hamy's proof that a centrally condensed barotrope cannot be stratified on concentric spheroids. Others are only stated, like Lichtenstein's theorem that a uniformly rotating and uniformly dense self-gravitating mass possesses an equatorial plane of symmetry. In chapter five, a number of approximate methods, perturbation and numerical, for constructing rotating configurations, are described. Chapter six is devoted to an up-to-date description of stability techniques, some of which find applications in chapter seven to obtain limitations on the allowable distributions of angular momentum.

The discussion of specific subspecialties begins with chapter eight (on meridional circulation) and concludes with chapter sixteen (on rotation in close binaries). In between there are chapters on the Sun's differential rotation, on the distinctions between uniformly and differentially rotating models, on the questions of collapse and fission, on a closer examination of the relation of the models to observations, on white-dwarf stars, on oscillation and stability properties, and on magnetic fields in rotating stars.

There exist some books and review articles covering some of these narrow areas in depth, and others covering broader areas superficially. Until the appearance of this book, there existed no reference attempting broad coverage in depth. Tassoul has filled this gap with a high standard of scholarship. In some respects,

his book has the appearance of a series of review articles on a variety of subspecialties, but it is much more. It is an account of the field, uniform in style and scholarship, and further unified by a particular viewpoint. The viewpoint is definitely from the theoretical side, and observational astronomers may find it uncongenial, although Tassoul has made consistent efforts to relate theory to observation.

The literature review is impressive. It must have required reading and digesting an enormous number of articles covering widely differing scientific viewpoints. The conclusions of these articles are not simply reported, but are compared, criticised, and, in some cases, synthesised. For example, in chapter eight on meridional circulation, a section of about ten pages is devoted to a critical review of the assumptions underlying the subject. Five principal assumptions are isolated and collected at the beginning of the section, and then examined one by one. This degree of thoroughness does not make light reading, but is certainly useful to anyone wanting to understand the subject in depth. Other examples of this kind of critical examination of assumptions, less formally organised, are found throughout the book.

Another, closely related, function of the book is to summarise the work of others. This is especially helpful when competing theories are summarised side by side. This is done, for example, in the cases of theories of the Sun's differential rotation (chapter 9), of the collapse and fission of protostars (chapter 11), and of the β -cepheid variables (chapter 14). It is inevitable in summarising conclusions from many subspecialties that some erroneous assertions must appear. But in reading with particular care the summaries of those areas I think I understand, I am principally impressed by their accuracy. The errors do not seem damaging. They are insignificant in comparison with the assistance the summaries provide to the otherwise unguided reader.

Nobody is perfect. Tassoul omits some topics that are currently exciting a lot of interest. The scope of a book like this does have to be limited in some way, and Tassoul's stated principle for doing so is to consider only topics of long standing from Newtonian (as opposed to general relativistic) mechanics. He does not discuss the widely accepted picture of pulsars as rotating neutron stars (because the subject is not of sufficiently long standing?) or X-ray binaries and black holes. He has apparently decided to direct attention only to those problems that have settled down to the point that their formulations (if not their solutions) are clear and not likely to change before the book gets into print. One can sympathise with this decision while recognising that it makes the book duller than it would otherwise be. It would not have required a deep excursion into the theory of general relativity to present some

of the current work and at least provide references. There is a precedent for this in the book, in the chapter on close binaries: the more questionable points are not discussed in detail, but references are given.

And there are some errors. I noted a succession of minor errors in chapter 6 on oscillations and stability. For example, the definition of stability given on page 116 fails to apply to conservative systems, as it requires departures from the unperturbed state to die away with time, rather than merely remain small. Next, the commutativity of the operators of Lagrangian perturbation and of material derivative is presented only in the linear approximation (page 119), whereas it holds quite generally. Perhaps this omission is excusable, as this is the presentation given in the usual reference, but it has the effect of propagating an erroneous impression. On page 122 it is asserted that the free boundary of a fluid mass is defined by the condition that the pressure vanish there. It is possible to get away with such a definition without making mistakes, at least in a number of special cases, but it is not the right mathematical description of the physical situation. The free boundary is defined as the mathematical (or

topological) boundary of the set where the density is positive, and the vanishing of the pressure there is a physical boundary condition, not a definition.

This frequency of errors is not typical. I noted a few more through the book, of the same degree of seriousness as those above: annoying, but not paralysing. Doubtless there are others I missed, but my net impression remains that the number of inaccuracies is remarkably small considering the scope of the book, and their negative contribution is negligible in comparison with the overall, positive contribution.

References are collected at the end of each chapter, related to sections within that chapter, and partially annotated, thus giving some guidance to the reader. There is an index of names, apparently quite complete, and an index of subjects, which I found incomplete.

This book should, and I feel sure will, become the standard reference for the subject matter covered. Anyone involved in the subject of rotating stars will simplify his life by having a copy on his shelf.

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Chinese chemistry and chemical engineering

Chemistry and Chemical Engineering in the People's Republic of China. Edited by John D. Baldeschwieler. Pp. 266. (American Chemical Society: Washington, DC, 1979.) Hardback \$15; paperback \$9.50.

THIS book is the report of a US delegation of chemists and chemical engineers which travelled widely in central and northeastern regions of the People's Republic of China during May–June, 1978. It is perhaps the most informative survey of chemical activities yet available. Chinese science, in modern form, began in the first half of this century and although at a rather preliminary level, seemed to be progressing slowly before 1965. In the following ten years, the influence of the 'gang-of-four' and its Cultural Revolution was serious, halting progress and destroying morale. Premier Chou-En-lai had talked in 1975 of the four modernisations, including science and technology, but only after the removal of the gang-of-four in 1976 could his principles be applied. In 1977–78 plans for re-orientations were announced by Chairman Hua Kuo-feng, and later by Vice-Premier Teng Hsiao p'ing, and Fang Yi, head of the Commission on Science and

Technology, then gave full details of a seven-year programme aimed at achieving parity with other big nations by the turn of the century.

There are three main sectors, the Chinese Academy of Sciences (CAS), the universities (under the Ministry of Education), and the Ministry of Production, all being coordinated and controlled by the Commission for Science and Technology (CST), whose head Fang Yi is a Vice-Premier, a Vice-President of the CAS, and a member of the Political Bureau. A few centres are run in whole or part by the provincial Government, and special divisions of the CST are dealing with energy, communications and industrial technology.

The CAS in Peking was greatly weakened in the Cultural Revolution, but its status has now been restored, and there are already some 90 research institutes across the country, covering the main fields of science, and branches of the CAS in Shanghai, Czechuan, Sinkiang and elsewhere. There are, at present, 14 Institutes for different aspects of chemistry (300–1000 staff) and scientists themselves are again playing a greater part in formulating policy, rather than the Revolutionary Committees, many old directors having been re-instated. The Chinese Academy of Medical Sciences (CAMS) has 13 Institutes in Peking and Shanghai, and links with hospitals and with the pharmaceutical industry. Research training courses for university graduates now operate in some of the Institutes.

The universities, too, were very disturbed by the gang-of-four. Foremost for chemistry, perhaps, are Peking, Shanghai (Fudan), Kirin and Canton, and for engineering Peking (Tsinghua) and Shanghai. Student numbers have increased considerably in the past two years. Entrance examinations have been re-introduced, giving first priority to intellectual ability, and there is to be far less time for political instruction. There is still a serious shortage of textbooks. The student/staff ratio is very low (average about 2.7), very different from that in European or American universities. Chemistry courses have been lengthened to four years, in which some mathematics, physics and applied science is included. Equipment for teaching is fairly good. Unfortunately, the effects of the Cultural Revolution were to create a sort of vacuum in the trained teaching staff, for the older members who had received training in Europe or America are now old or retired, and the middle-aged and younger groups were sent out to the Communes and have become dispersed. In spite of the intrinsic ability of the Chinese people, this shortage of adequate staff may not be corrected for many years. A few universities are trying to start advanced research training, but no degrees are to be given yet. Contractual applied research or actual production previously carried out in some universities has almost entirely ceased.

The pattern of industrial technology and research is complex, and under a combination of central and provincial control, as in the petroleum and chemical industries, and in the extensive research on catalysts. Scientific staff for the Taching oil development are mostly trained at the Peking Petroleum Institute, and the Harbin Petroleum Research Institute is still largely controlled by the provincial Government. Chinese students, after training, are prepared to be sent wherever their country needs them.

As might be expected, much of the current science and technology in China is directed towards practical needs. Impressive work is in progress on pharmaceuticals, especially those extracted from indigenous plants, traditionally used as medicinals; and on the synthesis of natural products and nucleic acids. These include peptides, hormones, prostaglandins, antibiotics and some anti-cancer and anti-bronchitis reagents. Much of this work is done in the Institutes of Organic Chemistry, Biochemistry and Materia Medica in Shanghai, and those of Materia Medica and Chemistry in Peking. The synthesis of insulin in the Institute of Biochemistry, Shanghai, some years ago was a famous achievement. Production of polymers, mostly the best known classes, has made good progress, with extensive research on improved catalysts for polymerisation. The Peking Institute of Chemistry has a large division on polymer research. There and in other Institutes, new

fluoropolymers, crown-ether type polymers, and others with functional groups having pharmacological or other useful properties, are being studied.

Excellent work is being done in the Institute for Ceramics and Metallurgy, Shanghai, including the growth of single crystals, ferrites, ceramics for the electronic industry and for high temperature equipment, diamonds, and crystals with special optical and electrical properties. Research in the petroleum and petrochemical fields is concentrated in Institutes at or near refineries or industrial complexes in some of the major cities. There is conventional work on the evaluation of crude oils, catalytic cracking and reforming of fuels, catalysts, lubricating oils and additives, engine testing and adhesives. Close contact between the Institutes and production plants is maintained. The manufacture of petro-protein using yeast is being explored, the products being tested as animal feedstuffs.

Rare earth ores are abundant in China, the elements being separated by ion exchange or through complexing reagents. Their possible use for the coating of fluorescence tubes, as converters of X-rays into visible radiation in medical equipment, and for catalysts is being explored.

By contrast, there is relatively little work on organometallic chemistry, molecular spectroscopy in relation to structure, quantum and theoretical chemistry, or reaction mechanisms and kinetics. Isotopes such as ^{18}O , ^{15}N , ^{13}C are being

produced for labelling and tracer work, but high-energy physics has not yet begun. The present computer facilities are limited. With a few exceptions, there is still shortage of newer physical instruments and techniques for structural determination and analysis, such as those for nuclear magnetic resonance, X-ray crystallography, mass spectrometry, electron or Mossbauer spectroscopy. There is much admirable home-made equipment of a less sophisticated kind, such as chromatographs. Work with lasers is still at an early stage, and studies with molecular beams have not yet started. All this is consistent with the policy of dealing with the more urgently practical applications first, but this situation may change quickly.

This book gives details of all places visited, names of scientific workers, charts of the organisation of science and technology, and the state of scientific information services in China. As regards both factual comments and the opinions expressed, it agrees closely with reports of Fellows of the Royal Society who have visited China in recent years. It is an admirable account for all interested in Chinese chemistry and chemical engineering.

H.W. Thompson

Sir Harold Thompson was formerly Professor of Chemistry at the University of Oxford and Foreign Secretary of the Royal Society. He has visited China three times: in 1962 and 1974 with delegations from the Royal Society, and earlier this year on behalf of the Great Britain-China Centre, of whose Executive Committee he is Chairman.

Trying to comprehend

The Nature of the Physical Universe: The 1976 Nobel Conference. Edited by D. Huff and O. Prewett. (Wiley-Interscience: New York and Chichester, UK, 1979). £12.75.

THIS volume collects together six lectures given at the twelfth conference supported by the Nobel Foundation at Gustavus Adolphus College, Minnesota. They are concerned with our understanding of the nature of the physical Universe, and with man's place in it. The level is intended to benefit an audience of intelligent laymen.

The first four lectures, given by scientists, are breathtaking in their scope. Viki Weisskopf begins at the microscopic level with the question "What is an Elementary Particle?". He quickly traces the history of man's exploration of matter, describing the energy level systems appropriate for atoms, for nuclei, and for nucleons. With such differing depths of exploration before us, a firm reply to this question is obviously difficult, but his final

answer is in terms of "conditional elementarity", the idea being that we can speak of elementary particles or not, only when we specify the energy resolution of our observations. The question whether quarks themselves may have structure is thus left open, for future research to settle. It is indeed a most pertinent question, now that we recognise 15 varieties of quark (5 flavours, each with 3 'colours'), since the discovery of the u particle in 1978. At present, we have no evidence for any structure, or finite size, for these quarks, but this situation may change when we can experiment at much higher energies and momentum transfers.

This story is then taken on by Murray Gell-Mann, who speaks about the new quantum number of 'colour', and about the gauge theory which has been built on it, known today as quantum chromodynamics (QCD). But there are also the 'leptons', the electron and its heavier counterparts, the μ and τ leptons, and the three neutrinos corresponding to them. Gell-Mann describes the programme of unification, recently successful in uniting electromagnetic and weak interactions to form the "electro-weak interaction"; with its next goal the

inclusion of the hadronic forces with them, he remarks that "this grand design has been for a long time our most cherished dream, and we now seem to be rather close to it". This situation would change suddenly, if quarks were found to have substructure.

Steve Weinberg asks "Is Nature simple?". His emphasis is on symmetries, not only the symmetries of space-time known from our daily life, but also the so-called internal symmetries, associated with charge, flavours, colour, and so on. For these latter, he introduces the notion of gauge symmetries, which imply the existence of gauge fields, with corresponding quanta; these gauge symmetries then govern the interaction of the gauge quanta with quarks and leptons. In this sense it is the gauge symmetry of colour which requires the existence of a gluon field, the gauge field for colour, which carries colour itself.

Most of the internal symmetries which we have known in elementary particle physics have turned out to be only approximately true, and, as a consequence of gauge theories, Weinberg now believes that "all these approximate symmetries can be, and in fact are being, understood as dynamical accidents". His remark is made specifically for flavour symmetries and it is a remarkable conclusion today, when one recalls what a great step forward the SU(3) unitary symmetry seemed to be in the mid-1960s. This has been a remarkable change in our point of view, which has dawned on most of us only quite recently, long after Weinberg's lecture. His remark applies even to parity conservation, allowing the possibility that particles with left-hand spin may have physical properties different from their right-hand counterparts. Weinberg concludes that "it is probably too early to try to think of drawing any philosophical lesson from discoveries in physics".

Fred Hoyle's lecture is concerned with the development of the Universe, right from the first moments of the 'Big Bang'. His emphasis is on the abundances of elements today, and on the conditions which must have existed to give rise to them. He draws our attention to the observation of simple organic molecules embedded in grains of meteoric dust, seeing in them a possible interstellar origin for life, arising even before our own Solar System formed. He traces out the nature of our chemical environment and leads us to the question "What does this tell us about the long-term future of our species?" These constraints imply a number of unpleasant conclusions concerning our very immediate future, for we cannot expect "to enjoy the great luxury of living in a hydrocarbon era" for many more generations. However, if population limitation were to come soon, and we could reach zero population growth, then "nothing concerning the future of our species would look unfavourable".

The last two lectures are rather

philosophical in style. Stanley Jaki of Seton Hall University lectured under the heading "The Chaos of Scientific Cosmology". His remarks about its history interested the reviewer, as we scientists hear rather little concerning older theories about the Universe, refuted by later evidence. It is odd to hear the views once accepted as reasonable by eminent scientists whose names have become law in the areas of their own scientific success. However, it is not clear whether the chaos Professor Jaki is speaking of is the chaos of this history of cosmologies, or whether it is the chaos of matter within the Universe. In his view, "the primeval chaos of fundamental particles" is connected somehow with its unreliable mathematical underpinning, unreliable because Gödel's incompleteness theorem removes any hope of demonstrating the consistency of any such mathematical system within itself. So he advises caution concerning any beliefs about scientific cosmologies or any implications they may suggest for Mankind.

The final lecture was given by Hilary Putnam, who discussed three areas lying outside present-day science: the domains of objective values, of freedom of choice, and of rationality. He rejects the notion that ethics are the result of instinct or conditioning. He considers whether our impression of free will results only from Chance but concludes that we have so little understanding of probability that this line of thought can offer no hope for any enlightenment. His opinion is that the selection pressures of evolution cannot account in full for the development of

ethics and rationality, an understandable view with which we will all have much sympathy. On the other hand, our ability to develop Science, and to comprehend and control our Universe by discovering physical laws and understanding how to use them for prediction, could well have arisen as a by-product of natural selection, in the reviewer's opinion. The situation does not call for the existence of any selection pressures directed specifically towards scientific activity. Also, our development of Science is not the result of testing random hypotheses, nor is it the end result in a chain of mathematical logic, the possibilities considered by Professor Putnam; it lies somewhere between, guided by the mind's ability to react to needs, to see analogies, and so to relate things apparently rather different, all characteristics which may simply be by-products of the survival value of rationality.

To this reviewer, the contrast between the earlier and the later lectures in this series illustrates well the enormous gulf between scientists and philosophers today, a gulf which is probably as large as it has ever been. Scientists have clarified large areas of human experience, in their analysis of the functioning and the forces of the physical world around us. This has enabled us to plan and to build, and to control great forces, and no doubt these abilities will grow much greater in the future. So it seems to a scientist a strange question, to be asked what fundamental justification there can be for the validity of his work, when he sees its products operating successfully and developing further, day by day. On the other hand, as Weinberg indicates, most physicists today would consider it unwise to seek within Science the proper basis for philosophy. Science is pragmatic, and we have seen enormous changes of viewpoint within the period of our own lives. All that was established before remains valid through these changes, of course, while becoming only a small part of the greater whole which has subsequently emerged and which continues to emerge. The relationships between scientific truths, and the perspective in our view of them, naturally continue to change as the picture becomes both larger and more detailed. As further knowledge is added, the patterns we see develop and come to have different meanings, and we must change our views in accord with them. Perhaps all this says is that we scientists no longer consider ourselves to be natural *philosophers*, seeking an absolute and final understanding, but see ourselves simply as men trying to comprehend, and to use for further discovery and comprehension, the forces acting within our Universe.

R.H. Dalitz

Errata

●In the review of *Ludwig Wittgenstein: Remarks on the Foundations of Mathematics* (see *Nature*, 281, 242; 1979), point 39 in the series of quotations from the work should have read:

39. The proposition ' $a=a$ ', ' $p \supset p$ ' ['If p , then p '], 'The word "Bismarck" has 8 letters', 'There is no such thing as reddish-green', are all obvious and are propositions about essence: what have they in common? They are evidently each of a different kind and differently used. The last but one is the most like an empirical proposition. And it can understandably be called a synthetic *a priori* proposition.

It can be said: unless you put the series of numbers and the series of letters side by side, you cannot know how many letters the word has.

Also, Michael Dummett, the reviewer, is Wykeham Professor of Logic in the University of Oxford, UK.

●In the review of *Handbook of Geochemistry* (see *Nature*, 281, 242; 1979), line 6 should have read "Part 5 is also by far the largest to appear,".

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